A COMPARISON OF POLYCYCLIC AROMATIC HYDROCARBON MOBILIZATION FROM ENVIRONMENTAL MATRICES

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Abstract

A method has been developed to analyse PAHs in solid environmental matrices using an *in-situ* PFE-GC-MS method. The method involves the use of 2 g of alumina as the *in-situ* clean-up sorbent, in order to remove interferences and impurities in the soils that could contaminate the instrument. By using this method, samples from two sites have been analysed for PAHs content, specifically (i) soils from a contaminated former Tar Works site, and (ii) urban road dust from Newcastle upon Tyne, UK. It was found that particle size was a significant parameter in both cases, showing a higher concentration (from 9 to 1404 mg/kg in the Tar Works, and 0.5 to 95 mg/kg in the road dust site) in the smaller grain size (< 250 μm); this is important when considering the ingestion exposure pathway as smaller particles are more likely to be ingested by children via hand-to-mouth behaviour. In addition, the source of the PAHs was investigated in the anthropogenically contaminated areas; it was found that pyrogenic sources (higher molecular weight PAHs, 4-5-6 rings) of PAHs were significantly more abundant compared to petrogenic sources (lower molecular weight PAHs: 2-3 rings). Generally the lower molecular weight such as naphthalene, acenaphthene, acenaphthylene and fluorene were found in lower concentration than fluoranthene, pyrene and other higher molecular weight PAH. In the case of the Tar Works lower molecular weights PAH were showing individual PAH concentration below 50 mg/kg whereas higher molecular weights were showing individual concentrations up to 270 mg/kg. The same trend was observed in the road dust samples, and was clearly identified by using ratios of PAH concentration to demonstrate dominance of pyrogenic sources. In this latter case, the pyrogenic sources were clearly identified as vehicle exhaust. However, other sources were identified such as the road pavement and the tire debris as potential sources of PAHs in urban areas. In the former case the PAH distribution was attributed to the locations of the chemicals productions areas in the former industrial site. The mean daily oral intake was used as an estimate of the

environmental health risk from the sites; values of PAH intake were determined based on the PAH individual concentration and compared against known values. Risk was often present for pyrogenic PAHs in road dust and soil samples. Further investigation of the environmental health risk was realized using a physiologically-based extraction test on soil samples from the former Tar works; the results, using a fed-version of the test, showed elevated bioaccessibilities of PAHs, mainly due to the presence of food and the lipophilicity of PAHs, however other PAH properties could influence their individual mobilizations such as the molecular weight, the ring number and the liquid-to-soil ratio.

It was noticed that the risk can be evaluated differently and can show different conclusions depending on the risk assessment chosen. Overall, the determination of PAHs in environmental soil and urban dust samples has highlighted the necessity to assess the potential impact on human health of their presence. The use of the fed-version of the physiologically-based extraction test is one tool that could be used to assess the environmental health risk to humans. This tool was shown to be robust using an inter-laboratory study, as values for total PAH content and bioaccessible fractions were within the same acceptable range.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others. The work was done in collaboration with the British Geological Survey, Keyworth

Name:

Signature:

Date:

Chapter 1: PAHs exposure in environmental matrices

1.1 Introduction

The pollution of the environment, and more particularly soils, has started to be a major concern for the public and scientists 40 years ago with events such as the Love Canal in the United States, Lasalle in Canada and Lekkerkerk in the Netherlands, which were involving serious controversy (Jacquet, 2007). Following those events, soil pollution became an increasingly concern in media, therefore entering a debate implying environmental, social, economic and public health issues. Discussions have lead environmental specialists and the government to (i) create norms and rules to avoid further pollution of those types, to (ii) evaluate the pollution levels on specified sites, and to (iii) remediate the other sites around the world that could have been polluted through various human activities (Jacquet, 2007). Indeed, numerous sites may have been contaminated since the beginning of industrialization, however it is only after those controversial events, that an interest in contaminated land has been manifested (Jacquet, 2007). Therefore, it can be expected that nowadays a large number of unknown contaminated sites have to be monitored.

A site can be characterized as contaminated when the pollution can lead to a risk to human and the environment. Various types of contaminations of solid environmental matrices generally exist (Rogge *et al.*, 1993; Jacquet, 2007) such as leaks occurring during the transport and storage of raw materials, agricultural practices leading to the use of contaminants to protect crops, atmospheric emissions, former and actual industrial sites, domestic emissions, vehicle exhaust, and materials (e.g: pavement, tyre debris) that can involve leaching of contaminants into matrices. Polluted sites can represent a risk for human health because of the direct contact between soils and people activities. Toxic substances can migrate gradually towards the supply sources of drinking water, or infiltrate houses via cracks, and can be transferred into plants (Jacquet, 2007). In the case of urban soils, it should be said that, due to the increased

activities in urban areas, these soils are getting more polluted, therefore being also a major environmental concern (Okorie, 2010). Street dust can be very harmful to human as dust particles can also easily become airborne through vehicular traffic (Rogge *et al.*, 1993; Miguel *et al.*, 1999; Liu *et al.*, 2007), thus possibly entering in contact with human via the respiratory tract.

There are different exposure pathways to environmental matrices which will indicate how a pollutant can enter in contact with human (Pumlee *et al.*, 2003; Sherwood, 2007). One important parameter in any exposure pathways is the particle size of the matrix, which will indicate when the matrix will be more easily in contact with human receptors (Pumlee *et al.*, 2003; U.S Environmental Protection Agency, 2008). After estimating the concentration of pollutants in a matrix with an analytical method, the levels are compared with Soil Guideline Values (SGVs) in order to evaluate the potential risk. For instance for PAHs, the values found in a solid environmental matrix such as soil will be compared with available SGVs in order to evaluate the risk, bearing in mind that SGVs for PAHs have not yet been released by the Environmental Agency (Smith *et al.*, 2007).

This chapter will firstly describe (1) the three different exposure pathways that exist in the environment, then (2) the importance of particle size when considering these three pathways of exposure. A description (3) of the CLEA (Contaminated Land Exposure Assessment) model will be made, before describing (4) soil guideline values for PAHs and (5) PAH occurrence in the environment and their properties.

1.2 Exposure pathways to pollutants and environmental matrices

Exposure assessment is “the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, pathways, routes, and the uncertainties in the risk assessment” (Environment Agency, 2009c).

Additionally, even if a contaminant is present at high concentration in a matrix, when there is no exposure, there will be no possibility to involve a risk (Environment Agency, 2009b). Risk is inevitably linked to exposure, consequently, the exposure pathway is as much important as estimating concentration of a pollutant in a matrix (Environment Agency, 2009b). Indeed, in order to assess the risk from pollutants in the environment, the recent models developed by environmental agencies and other organizations involve the use of the pathway of exposures between human and the pollutants contained in the environmental matrices (Plumlee *et al.*, 2006; U.S Environmental Protection Agency, 2008). The contact can be made through different routes such as (Environment Agency, 2009c):

(i) Via ingestion through the mouth.

(ii) Via inhalation through the nose and mouth.

(iii) Via absorption through the skin.

The first case, which is the one considered in this entire study, involves principally young children because of their hand-to-mouth behaviour, with objects on the floor (Versantvoort *et al.*, 2004). These pathways of exposure are included in a more general description of the links between a pollutant in a matrix and the humans and environment. As part of the Environmental Protection Act, there is a concept of pollutant linkage which implies three essential elements to any risk (Environment Agency, 2009d):

(i) A source: a substance, contained in a matrix and which is classified as dangerous for a particular receptor

(ii) A receptor: an individual or element that can be threatened by the contaminant.

(iii) A pathway: a link between the receptor and the contaminant, which permits the contaminant to be in contact with the receptor and conversely, depending on the pathway of exposure.

This pollution linkage describes the presence of a potential risk, assuming the three are linked together (Environment Agency, 2009d).

1.3 Importance of particle size

The particle size is important in the three various pathways of exposures. According to the size of the particle of the solid environmental matrix (i.e. soil or dust), it will be ingested through the mouth, inhaled *via* the nose or mouth, or absorbed through the skin. Particle sizes below 250 µm are generally accepted as the size where particles can easily adhere on the skin (Bornschein *et al.*, 1987; Rodriguez *et al.*, 1999; US Environmental Protection Agency, 2000). Therefore, this particle size needs to be considered when working on the potential ingestion or absorption of chemicals through solid environmental matrices. Secondly, the finer particle size < 63 µm and more particularly $PM_{2.5}$ (Particulate Matter 2.5 µm) and PM_{10} are generally considered in the inhalation pathway because they can easily become airborne (Miguel *et al.*, 1999). Several studies on road dust and soils have shown that particle size has an importance in the distribution of PAHs. Generally, an increase in concentration was observed as grain size was decreasing, which is important in the study of the ingestion of solid environmental matrices (Dong *et al.*, 2007; Zhao *et al.*, 2009). Indeed, if a higher PAHs content could be ingested via soil or dust with finer particle size, it could represent a higher risk for human health.

1.4 The Contaminated Land Exposure Assessment (CLEA) model

The CLEA is based on a model which tries to describe precisely the different ways of exposure from chemicals present in environmental matrices to human living, working and/or playing on contaminated land, over significant period of time (Environment Agency, 2009d). Considering the ingestion of soil, the risk is currently based on the possible ingestion of 100 mg/day of soil or dust by a children aged between 1 and 6 years. This value has been recently established and used by the USEPA and the RIVM (Oomen *et al.*, 2006; U.S Environmental Protection Agency, 2008), and those

values are generally used nowadays when considering the study of the possible ingestion of contaminants via soil or dust. 150 or 200 mg/day of soil or dust ingested per day represent a realistic worst case scenario (Otte *et al.*, 2001). In order to respond to problems due to contaminated land and pollutants in environmental matrices, environmental agencies and local authorities are developing models that industrials and researchers could use so as to define a site as contaminated or not and if remediation would need to be realized in the future. For example, remediation can help a land owner to increase the utility and value of their land (Department for Environment Food and Rural Affairs, 2008).

Section Part 2 of the environmental protection act 1990 (Department for Environment Food and Rural Affairs, 2008) defines contaminated land "as any land which appears to the local authority in whose area the land is situated to be in such a condition, by reason of substances in, or under the land, that significant harm is being caused or there is a significant possibility of such harm being caused". In this particular domain, harm means a potential hazard or threat to the health of human, animals and plants. Indeed, this act provides a statutory guidance which defines what a significant harm means, and guide local authorities so as to explain if there is a significant possibility of significant harm (Department for Environment Food and Rural Affairs, 2008). This guidance explains firstly that significant harm to human health includes various forms of diseases or dysfunctions that could affect human health, secondly, that the amount of pollutant to which a person might be exposed would represent an "unacceptable" intake or "unacceptable" direct bodily contact (Department for Environment Food and Rural Affairs, 2008).

To help finding if the level of a contaminant can induce significant harm or the possibility of significant harm, the Contaminated Land Exposure Assessment supplies with a device where risk assessors can enter contaminant levels, estimates and assumptions about the factors that influence chemical exposure on a site (Environment Agency, 2009e). This tool provides information on the potential human

health risk when in contact with contaminated soils (Department for Environment Food and Rural Affairs, 2008): firstly (i), it permits to establish if a pollutant in soil can be transmitted to human through different pathways of exposure such as ingestion, inhalation and skin contact, as described previously. Secondly (ii), it permits to estimate the exposure level of a contaminant which could induce a significant harm in the case of penetrating the human body. In this case, the assessor needs to choose an estimate that could induce significant harm as a result of long term exposure. Finally (iii), the tool will demonstrate if there is any possibility that the contaminant present in the soil would involve a significant harm for the human considered. This would be based on measurements, estimates and assumptions about the contamination levels on a specific site. By using this tool, risk assessors can evaluate the risks on a specific site, for a contaminant. In England and Wales, the estimation of the risks are based on the CLEA model, which involves comparison of pollutant levels with SGVs derived from HCVs (Health Criteria Values), and where a concentration below or at this value will involve minimal risk for humans (Environmental Agency, 2005). The CLEA employ estimates of exposure based on intake (i.e the amount of contaminant that can be in contact with the human, defined by mg/kg bw/day), rather than on the uptake (i.e the dose of contaminant that can potentially reach the systematic circulation) (Environment Agency, 2009b). Indeed, the HCVs are obtained mainly by using the intakes values, resulting from the contaminant content evaluation, using animals or humans and considering exposure to various matrices (Environment Agency, 2005); this first assumption explains that a contaminant might be taken up by the body from the matrix to the same extent as from the medium of exposure used in the study, to derive the oral HCV. This consideration could be wrong as soil contaminants for example can be sequestrated in the matrix, leading to lower the contaminant bioavailabilities (Environment Agency, 2009c). Each contaminant can be bound in the matrix differently, so that some substances can be more easily adsorbed into the ingested soils than with the medium used in the toxicology study (Environment Agency, 2005). Furthermore, the HCVs are

associated with two types of substances to establish the risk (Department for Environment Food and Rural Affairs, 2008) (Figure 1.1):

(a) The threshold substances which consider a level at and below there is no risk for human. The government describes this level as a value where there is no appreciable risk to human health. This threshold will be called the Tolerable Daily Intake (TDI) and is expressed on a bodyweight basis (mg/kg bw/day)

(b) The Non-threshold substances will involve risks at any level of exposure, this is described by the government as a minimal risk to human health. In this case an Index Dose (ID) will be required to define the risk.

The Mean Daily Intake (MDI) can also be used when other matrices than soils are considered for the exposition of humans, such as food, water, and air (Environment Agency, 2009b). The MDI is defined in units of mass per day (µg/day). Moreover, the intake will vary, depending on the fate and transport of chemical in the environmental matrix, because complex processes are involved inside the soil such as partitioning due to contaminants being (i) adsorbed into soil organic matter, (ii) dissolved in the interstitial pore water, (iii) isolated in the gas phase, and also persistence, and transport of targeted compounds from a matrix to another (Environment Agency, 2009c).

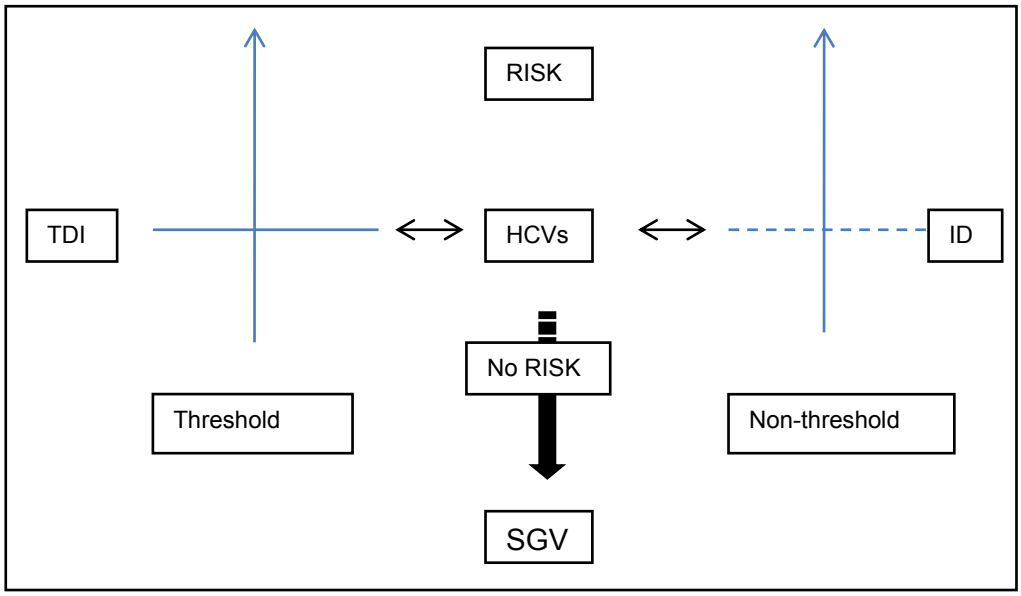


Figure 1.1: Description of the risk estimation based on the derivation of HCVs, considering threshold and non-threshold substances

1.5 Soil guideline values and PAHs

The environmental agency has not yet released SGVs for PAHs (Smith *et al.*, 2007; Environment Agency, 2009a), however some other documents establish generic assessment criteria between 0.83-2.1 mg/kg for benzo(a)pyrene in residential soils and allotments soils with organic matter ranging from 1 to 6 % (Nathanial *et al.*, 2009) . In other countries such as Denmark and Belgium, the threshold level for a unique PAH is generally fixed at 1 mg/kg (Cave *et al.*, 2010). This is confirmed by the Dutch environmental regulations which estimates 1 mg/kg as a level where there is a potential risk, and a value of 40 mg/kg for the total of 10 PAHs (VROM, 2000). In the UK the threshold value for total PAH content was fixed at 50 mg/kg for residential and domestic areas, but those values have not considered being up-to-date (ICRCL, 1987). It should be noted that in the majority of contaminated sites in the UK, values exceeds the GACs (Nathanial *et al.*, 2007).

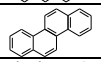
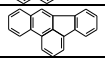
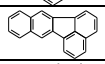
1.6 Occurrence of PAHs in environmental matrices

PAHs, which are part of a larger group called the persistent organic pollutants (POPs), are a type of components that can become easily airborne in the atmosphere, and will be distributed between both gaseous and particulate form, owing to their respective vapour pressures (Mostafa *et al.*, 2009). Persistence and hydrophobicity of such compounds will conduct them to stay in the solid environmental matrix for several years (Motelay-Massei *et al.*, 2004). Indeed, the hydrophobicity and stable chemical structure of those compounds, making them not very soluble in water, they will be adsorbed on soil particle and soil organic matter (Tang *et al.*, 2006). Therefore, the solid environmental matrices will act as a container for those pollutants. Soil has been reported to be the primary reservoir for PAHs (Tang *et al.*, 2006). This will involve significant risks for the environment in the case of contaminated agricultural soils and its corresponding trophic chain (Motelay-Massei *et al.*, 2004). More generally, POPs will be transferred from the natural or anthropogenic source, into the environment, such as natural waters, sediments, soils, and they will enter plant, vegetables, and

other food components (Hubert *et al.*, 2003). PAH are a group of organic compounds which are non-polar, hydrophobic, contain two or more fused benzene rings (Kim *et al.*, 2003), and are ubiquitous in the environment (Berset *et al.*, 1999). More than 100 PAHs can be found in the nature (Barranco *et al.*, 2003), but the US Environmental Protection Agency has only classified 16 of them as priority pollutants due to their occurrence, mutagenic and carcinogenic properties (Barranco *et al.*, 2003). They are listed in Table 1.1 with their respective potential harmful effect, partitioning coefficient, solubility in water, melting point, ebullition point, structure, mass, and potential harmful effects. This explains why a larger number of studies are involved into the characterization of those pollutants in the environment, which can be toxic to humans and have hazardous effects on soil organisms and plants (Ong *et al.*, 2003).

These compounds are formed through combustion within anthropogenic and natural processes. The former involves burning of fossil fuels, coal-derived, coke production, industrial processes (Graham *et al.*, 2006), the latter involving principally forest fires, volcanic activities and geochemical processes (Liguori *et al.*, 2006). They can also be present in food due to heat processes such as smoking, grilling and smoke drying (Liguori *et al.*, 2006), and in asphalt processing and use (Takada *et al.*, 1991; Mahler *et al.*, 2010). High concentrations of PAHs were found in sites where coal, coal-tar, or heavy petroleum distillates were produced or used, for example gas works, tar works, metal or bitumen production sites, and wood impregnation sites where creosote was used (Ong *et al.*, 2003). Those industrially contaminated sites are often situated close to human activities and houses, therefore requiring remediation (Ong *et al.*, 2003). This type of hydrophobic organic contaminants (HOCs) can enter the human digestion via the ingestion of solid environmental matrices, and therefore can be adsorbed into the gastrointestinal epithelium (Vasiluk *et al.*, 2008).

Table 1.1: Structure, empirical formulae and other properties of the 16 PAHs

PAH Structure	Empirical Formulae	PAHs	MS Ion for Quantitation	Risk group (IARC)[a]	Bpt (ºC)	Mpt (ºC)	Log K_{ow} [b]	Solubility in water at 25 ºC µg/kg
	C10H8	Naphthalene (NAP)	128	2B	218	80.5	3.35	31.7
	C12H8	Acenaphthylene (ACY)	152	ND	280	80-83	4.07[c]	39.3[c]
	C12H10	Acenaphthene (ACE)	154	3	279	96.2	3.92	3.42
	C13H10	Fluorene (FLU)	166	3	293	116	4.18	1.98
	C14H10	Phenanthrene (PHE)	178	3	340	101	4.57	1.29
	C14H10	Anthracene (ANT)	178	3	340	216	4.54	$4.5x10^{-2}$
	C16H10	Fluoranthene (FLUH)	202	3	250	107	5.22	$2.6\ x10^{-1}$
	C16H10	Pyrene (PYR)	202	3	360	150	5.18	$1.35x10^{-1}$
	C18H12	Benzo(a)anthracene (BaA)	228	2B	435	162	5.79	$5.7x10^{-3}$
	C18H12	Chrysene (CHY)	228	2B	448	255	5.98	$1.9x10^{-3}$
	C20H12	Benzo(b)fluoranthene (BbF)	252	2B	481	168	6.06	$1.4x10^{-2}$
	C20H12	Benzo(k)fluoranthene (BkF)	252	2B	480	217	6.06	$4.3x10^{-3}$
	C20H12	Benzo(a)pyrene (BaP)	252	1	495	179	6.00	$3.8x10^{-3}$
	C22H12	Indeno(1,2,3-cd)pyrene (IDP)	276	2B	536	164	6.40	$5.3x10^{-4}$
	C22H14	Dibenzo(a,h)anthracene (DBA)	278	2A	524	267	6.86	$4.0x10^{-4}$
	C22H12	Benzo(g,h,i)perylene (BgP)	276	3	500	222	7.10	$3.0x10^{-4}$

[a] Evaluation of risk according to International Agency for Research on Cancer (IARC), 1=Carcinogenic to human; 2A= Probably carcinogenic to human; 2B= Possibly carcinogenic to human; 3= not classifiable as to its carcinogenicity to humans; 4: Probably not carcinogenic to humans (Li *et al.*, 2010)
[b] (Lu *et al.*, 2009)
[c](Tang *et al.*, 2006)

PAHs can also create further metabolites when entering the digestion system potentially causing DNA damage, chromosomal mutation and increased risk of leukaemia in childhood (Liguori *et al.*, 2006). Generally, benzo(a)pyrene is chosen as an indicator of the total 16 PAHs (Liguori *et al.*, 2006), and has been identified by the Environment Agency (UK) and European Community as a carcinogenic marker substance, and as the most carcinogenic of all PAHs (Vasiluk *et al.*, 2008). There is a lot of toxicological evidence on this particular compound, showing that tumours have been produced in several kinds of animals, following administration of benzo(a)pyrene through various pathway of exposure (Health Canada, 1986).

The sources of PAHs are generally grouped in two different types: one considering PAHs resulting from anthropogenic sources and the other PAHs resulting from natural sources. Generally, the higher molecular weights PAHs are dominated by the combustion of fossil fuels and vehicle exhaust whereas low molecular weight PAHs are generally dominated by natural sources: those two different types of sources are defined by the terms pyrogenic for the higher molecular weight, and petrogenic for the lower molecular weight (Yunker *et al.*, 2002). Those two types have been used extensively in the literature in order to compare distribution of the 16 PAHs in soils and road dust (Yunker *et al.*, 2002; Wang *et al.*, 2009). Usually, a high concentration of higher molecular weight indicates anthropogenic pollution whereas a high content with low molecular weight PAHs indicates natural pollution. Moreover, one study has shown that PAHs in urban areas can be more than ten times higher than those in rural areas (Lu *et al.*, 2009). As described in the Figure 1.2, the potential sources of PAHs in street dust in an urban site are atmospheric deposition, vehicle exhausts, tyres debris, road surfaces, brake lining and cigarette ash.

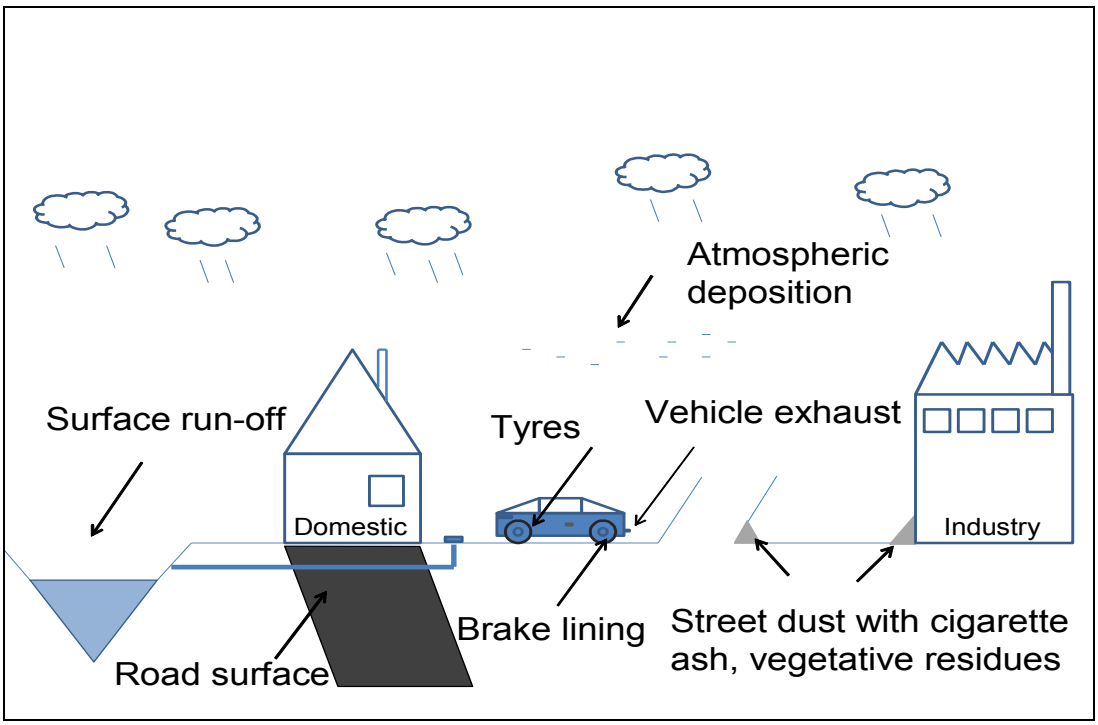


Figure 1.2: Sources of PAHs in an urban site

1.7 PAHs properties

In order to clearly understand the behaviour of PAHs in the environment and in solid or liquid environmental matrices, it is important to know the properties of those compounds, which can indicate why a specific trend or behaviour is observed. As described in the Table 1.1, the low molecular weight PAHs are more volatile than the higher molecular weights. For example, when comparing the lowest and highest molecular weight, naphthalene has a boiling point at 218 ºC whereas dibenzo(a,h)anthracene has a boiling point at 524 ºC, The contrary is observed when considering the solubility. Solubility in water at 25 ºC is decreasing when increasing the molecular weight of PAHs. PAHs are known to have very low solubility in water as they are hydrophobic compounds, which is further confirmed by the partition coefficient (Log K_{ow}), which are increasing with augmentation of PAHs molecular weight. As described in the precedent paragraph, benzo(a)pyrene can be harmful for human health, and it has recently been established as carcinogenic to human (Group 1) (IARC, 2010), as in the past it was classified as probably carcinogenic to human

(Group 2A) (IARC, 1983). This last update on the carcinogenicity of PAHs has also changed the risk group of benzo(a)anthracene and chrysene from group 2A and 3 respectively, to 2B for both compounds (Table 1.1) (IARC, 2010). According to the IARC's guidelines on risk assessment, the PAHs can be classified as carcinogenic to humans (Group 1), probably carcinogenic to human (Group, 2A), possibly carcinogenic to human (Group 2B), not classifiable as to its carcinogenicity to humans (3), and probably not carcinogenic to humans (Group 4) (IARC, 2010). With a good understanding of the PAHs properties, the evolution of PAHs distribution in environmental matrices can be explained, as interaction of those organic compounds with particles of soils or in an aqueous phase can be described. However, an understanding of the properties is not sufficient to characterize the risk as it is only related to the levels of contaminants in a media. Therefore, to evaluate the risk, the level of pollutant needs to be evaluated using appropriate analytical methods, but most importantly they need to be compared with specific values or guidelines that can inform the risk assessor where a hazard is likely to be present or not.

1.8 Conclusion

This first chapter introduces the issue on how to deal appropriately with level of contaminants, especially PAHs, in environmental matrices, when risk assessment needs to be realized. Currently, the risk assessment is based on these assumptions and regulations established by environmental agencies. However, there is ongoing work on how to improve the way the risk is estimated. For instance, estimation of bioaccessibilities using *in vitro* gastrointestinal tests is a way to refine the risk assessment already being used by the CLEA model. Indeed, the bioaccessibility gives information on the intake of pollutant through ingestion of solid environmental matrices, and is calculated, based on contaminant mobilized in the gastrointestinal fluids. Metals have been largely investigated using these methods (Ruby *et al.*, 1996; Rodriguez *et al.*, 1999; Gron *et al.*, 2003; Schroder *et al.*, 2004; Drexler *et al.*, 2007), and nickel or PAHs have been less explored (Gron *et al.*, 2003; Pu *et al.*, 2004). To

validate these values, the procedure requires validation by comparison with *in vivo* studies that are not always available for bioaccessibility studies, especially with PAHs. An other way to validate those studies is to realize inter-laboratory evaluations using physiologically-based extraction tests, which have started to be done in the recent years (Versantvoort *et al.*, 2004; Cave *et al.*, 2010).

This model requires an exhaustive understanding of the mechanisms that control human digestion and the possible interaction between contaminants and human organs inside the gastrointestinal tract, when ingested via solid environmental matrices. It also requires an overall understanding of the methods to isolate PAHs from complex solid and liquid environmental matrices, in order to identify risk and contaminant mobilization with as little uncertainty as possible. By understanding and applying these analytical tools, the risk assessment could be, on the one hand, estimated and on the other hand, improved.

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Chapter 2: PAH mobilization in the human gastrointestinal tract

2.1 Introduction

As ingestion is one of the main pathways of human exposure to pollutants from environmental matrices, scientists are trying to model human digestion of those matrices to estimate potential risks**.** As described in the previous chapter, ingestion of soil mainly occurs involuntarily via hand-to-mouth behaviour, and involves principally young children (U.S Environmental Protection Agency, 2008). Obviously, *in vivo* studies are the first way to estimate the risk through those behaviours. However, these types of studies involve ethics, financial issues, and physiological divergences between human and the contaminant behaviour inside the matrix (Scoof, 2004). These constraints have led to the development of *in vitro* gastrointestinal models, on the one hand, to estimate the bioaccessibility of contaminants in various matrices, as an indicator of *in vivo* bioavailability, and on the other hand, to refine the risk assessment in contaminated land management (Environment Agency, 2005).

Several *in vitro* gastrointestinal models have been investigated since the 1990s in order to mimic the human digestion of several contaminants such as metals and organic compounds in matrices such as food, soils and toys (Oomen *et al.*, 2002). The tests are based on the medical physiology of the gastrointestinal tract. Three main compartments are involved in human digestion: (a) the mouth allows grinding and masticating the food ingested with saliva, (b) the stomach, stores food and initiates digestion by churning food and secreting proteases and acid, and (c) the intestine, is made of two different compartments, the small intestine and the large intestine (the colon). The former, permits digestion and absorption of nutrients, while the latter permits to store indigested remnants before defecation (Intawongse *et al.*, 2006; Sherwood, 2007). The colon plays a significant role in degrading nutrients with bacteria (Petersen, 2007). The PAHs can be degraded by microbial contact and will potentially form new PAHs-based molecular structures or metabolites which can be

harmful for humans (Roberts *et al.*, 2000; Van de Wiele *et al.*, 2004). As evidenced by medical physiology, nutrients absorption will mainly occur in the small intestine. Therefore, some *in vitro* gastrointestinal tests do not include the saliva part, and focus often only on the gastrointestinal compartment (Hack *et al.*, 1996; Van de Wiele *et al.*, 2004), because absorption of nutriments will occur via the epithelial cells lining the small intestine.

In previously published studies, a good correlation has been found for selected metals such as lead and to some degree for arsenic and cadmium between *in vitro* bioaccessibility and *in vivo* bioavailability (Ruby *et al.*, 1996; Rodriguez *et al.*, 1999; Gron *et al.*, 2003; Schroder *et al.*, 2004; Drexler *et al.*, 2007). Concerning nickel and PAHs, only very few *in vivo* bioavailability studies and comparison between *in vitro* and *in vivo* data have been published (Gron *et al.*, 2003; Pu *et al.*, 2004; Gron *et al.*, 2007). It needs to be kept in mind that the bioaccessibility of a contaminant represent its mobilization into the gastrointestinal juices (intake or external exposure), whereas bioavailability represents the potential absorption of the contaminant into the systemic circulation (uptake or internal exposure) (Environment Agency, 2005; 2007). The *in vitro* gastrointestinal model explored in this project is the UBM (Unified BARGE Method) developed by a group of researcher within the Bioaccessibillity Research Group of Europe (BARGE) (Cave *et al.*, 2006). Before agreeing to a common procedure, the BARGE has compared several *in vitro* gastrointestinal tests, in several laboratories around the world (Oomen *et al.*, 2002).

Recently, the UBM has been modified, based on the studies from the National Institute for Public Health and the Environment (RIVM), with fed-based *in vitro* gastrointestinal tests, in order to produce a model simulating the ingestion of soil and food, considering a child aged between 1 and 6 years old (Versantvoort *et al.*, 2004). The method is called the Fed ORganic Estimation Human Simulation Test: FORES(h)t) (Cave *et al.*, 2010). This test is particularly adapted to the evaluation of PAHs bioaccessibilities in soils, because firstly it is more realistic, as food is part of

digestion; Secondly, it involves increased mobilization of PAHs through bile micelles and fat constituents, increasing the human health risk via their absorption into the surface of the cells covering the inner layers of the small intestine microvilli (Hack *et al.*, 1996; Gron *et al.*, 2003).

Now that an agreed procedure to realize a fasted *in vitro* test has been developed, the new step forward is to demonstrate that those models are reproducible and robust by doing inter-laboratory evaluations (Cave *et al.*, 2010). Some have already been realized for various contaminants in different matrices (Versantvoort *et al.*, 2004; Wragg *et al.*, 2009). When the robustness of the method will be demonstrated, the aim would be to use the most suitable model in commercial laboratories, in order to assess the human health risk, as currently there is only one inter-laboratory study on the FORES(h)t (Cave *et al.*, 2010). There is also a quality issue on the non-existence of a reference material for bioaccessibility studies (Environment Agency, 2007). Furthermore, the bioaccessibility tool seems a more suitable way to measure the risk, than the measurement of pollutant levels in environmental matrices compared to soil guidelines values.

This introductory chapter will firstly deal with (1) the medical physiology of the gastrointestinal tract. Then (2), a presentation of physiologically-based extraction tests will be made with an attention on the reasons to develop such a model. The various parameters (3) influencing the mobilization of pollutants during digestion will be described. The different steps (4) towards the elaboration of a fed state of that model will be presented. Issues of quality and reproducibility (5) will be discussed as part of the validation of the analytical protocol, related to this model. Finally (6), the fed model will be described as a promising and useful tool to refine the risk assessment, especially for polycyclic aromatic hydrocarbons.

2.2 Physiology of the gastrointestinal tract

In order to mimic human digestion it is indispensable to understand the various complex mechanisms that occur inside the human gastrointestinal tract. The main steps in the digestion involve the mouth, the stomach, the small intestine, and the large intestine or colon which are part of the gastrointestinal tract (Figure 2.1). Numerous enzymes are involved allowing digestion of food, secretion and absorption. Indeed, the four basic digestive processes are motility, secretion, digestion and absorption (Sherwood, 2007). The digestive tract is a long tube that runs from the mouth to the anus and the all process can also be summarized in seven steps (Dean *et al.*, 2007): ingestion, mastication, deglutition, digestion, absorption, peristalsis and defecation.

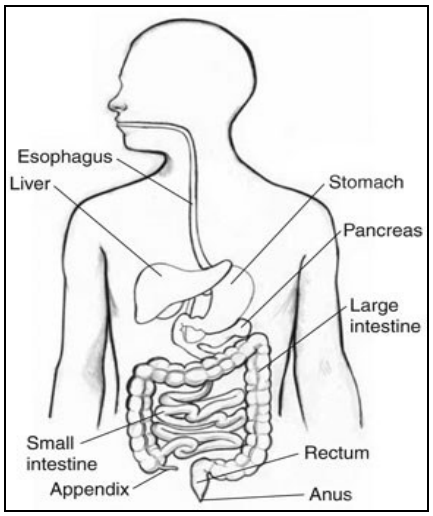

Figure 2.1: View of the gastrointestinal tract (NIDDKD, 2009)

In the mouth, food penetrates the digestive system where it is chewed, masticated and mixed with saliva to facilitate swallowing (Sherwood, 2007). The salivary enzyme, amylase, begins the digestion of carbohydrates. No absorption of nutrients occurs from the mouth and the entire process will only last a few minutes (Intawongse *et al.*, 2006). This can be explained by the fact that often the mouth compartment is not included in *in vitro* gastrointestinal tests. The nutrients are then transferred into the stomach *via* a tube called the oesophagus.

The stomach, a sac-like structure located between the oesophagus and small intestine, stores ingested food for variable periods of time until the small intestine is ready to process it further for final absorption (Sherwood, 2007). Gastric secretions into the stomach lumen include hydrochloric acid, which activates pepsinogen, denatures protein, and kills bacteria. Pepsinogen, after being activated, initiates protein digestion (Dean *et al.*, 2007). Carbohydrates digestion continues in the body of the stomach under the influence of the swallowed salivary amylase. Protein digestion occurs in the antrum of the stomach, where strong peristaltic contractions mix the food with gastric secretions, converting it to a thick liquid mixture known as chyme. Again, no nutrients are absorbed from the stomach (Sherwood, 2007).

The liver will then contribute to the secretion of bile, which contains bile salts, cholesterol and lecithin (Sherwood, 2007). Bile salts will be part of the digestion by forming micelles that will carry the fatty residues throughout the gastrointestinal tract until the layer of the small intestine (Gron *et al.*, 2003). Indeed, the micelles are constituted by long hydrophobic chains and hydrophilic heads which gathered together, form a spherical particle (Figure 2.2). The core of this micelle will be lipohilic and therefore attract all fatty components in the gastrointestinal tract, while the hydrophilic heads will permit to the micelle to circulate easily in the aqueous phase reaching the small intestine for absorption (Gron *et al.*, 2003) (Figure 2.2). The hydrophobicity of the fat matrix and of the micelle core is particularly important in the mobilization of polycyclic aromatic hydrocarbon which are lipophilic and non-polar compounds (Hack *et al.*, 1996; Oomen *et al.*, 2000). Monoglycerides and free fatty acids are the main components transported by micelles as a result of fat digestion (Hack *et al.*, 1996). When those compounds are not attracted onto the hydrophobic core of the micelle, they will remain in the aqueous phase (chyme), therefore not reaching the absorptive sites of the small intestine (Sherwood, 2007).

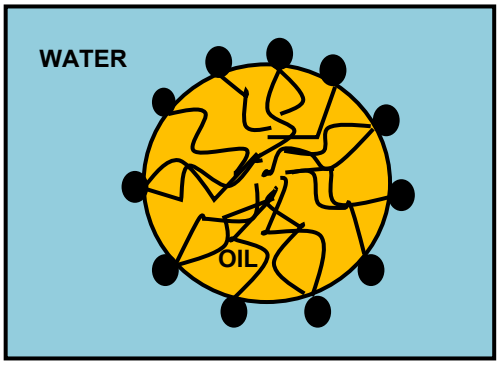

Figure 2.2: Description of a bile salt micelle

In the small intestine, brush-border enzymes complete the digestion of carbohydrates and protein. Fat is digested entirely in the small-intestine lumen, by pancreatic lipase (Sherwood, 2007). Absorption will occur through the fingerlike projections, covering the inner layer of the small intestine, more commonly called villi, which forms also the microvilli, a smaller version of those finger likes protrusions (Gron *et al.*, 2003). These surfaces will be responsible for the absorption of the resulting components of fat digestion (Sherwood, 2007). Finally, only a small amount of fluid and indigestible food residue passes on to the large intestine.

The colon serves primarily to concentrate and store undigested food residues until they can be eliminated from the body as faeces. No secretion of digestive enzymes or absorption of nutrients takes place in the colon, all nutrient digestion and absorption having been completed in the small intestine (Sherwood, 2007).

2.3 Development and design of an *in vitro* gastrointestinal test

Therefore, on the basis of human physiology, simulated gastrointestinal models have been developed. They are generally based on the different reagents and enzymes found in the saliva fluid (mouth), gastric fluid (stomach), bile fluid (intestine) and duodenal fluid (intestine). However, some tests include for example the colon or exclude the mouth (Oomen *et al.*, 2002; Van de Wiele *et al.*, 2004), some are very simple, whereas others are more complicated (Oomen *et al.*, 2002). In all cases they are trying to mimic the ingestion of a matrix such as soil or food and the effect on the

mobilization of the pollutants inside the gastrointestinal fluids, using different parameters and varying amount of soil or food depending on the conditions established for the model. To simulate as precisely as possible the gastrointestinal tract, the temperature of the extraction should be fixed at body temperature 37°C, and in order to mimic the peristaltic actions of the oesophagus, the extractions has to be performed by shaking or agitation (Dean *et al.*, 2007). Several ways of agitation have been reported such as end-over-end, mechanical stirring, peristaltic movements, argon gas dispersion and head-over-heels (Dean *et al.*, 2007).

After extraction, the compounds need to be isolated from a matrix containing generally biological fluids, water, food and soil particles. Suitable methods of extraction need to be used to simplify this complex matrix. Typically, techniques used can be filtration, centrifugation, saponification and extraction methods such as liquid-liquid extraction, solid phase extraction, solid-phase micro-extraction, stir-bar sorptive extraction, micro-extraction by packed sorbent, employed generally with liquid matrices (Dean, 2009). After isolation, analysis can be realized using analytical instruments such as, GC-ECD, GC-MS, HPLC-UV depending on the matrix and on the compounds analysed (Intawongse *et al.*, 2006; Dean *et al.*, 2007).

The pollutant concentrations in the resulting aqueous phase are then measured as the bioaccessible fraction and are defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract, and thus become available for intestinal absorption (Environment Agency, 2005). Bioaccessibility only provides an estimation of the fraction of contaminant in soil potentially available for absorption whereas the bioavailability will represent solubilisation and absorption inside the gastrointestinal tract (Environment Agency, 2005). The calculation of the bioaccessible fraction is a way to estimate bioavailability, but a compound that is bioaccessible will not be automatically bioavailable. This involves an understanding of the mechanisms of absorption of a particular contaminant into the systemic circulation.

The simulated *in vitro* gastrointestinal tests can be described either as static or dynamic: when a model is static, it consists in simulating the exposure of the samples with the fluids from the gastrointestinal tract, whereas a dynamic model will mimic the transfer of the samples within the various gastrointestinal fluids (Intawongse *et al.*, 2006). The dynamic tests are not as numerous as the static models, and the static models are generally more simple to use (Intawongse *et al.*, 2006). The main simulated *in vitro* gastrointestinal tests that have been developed in the last years have been summarized in a study (Oomen *et al.*, 2002), which is synthesized in Table 2.1. This table describes the main differences between the tests, such as the compartments of the gastrointestinal tract used, if food is added and if *in vivo* studies have been realized, and finally if the simulated test is static or dynamic.

The variation between the types of reagents used, the amount of soil, the incubation time, the soil-to-solution ratio, have led to differences in the resulting bioaccessibilities from those different models. However, this works was realized towards the elaboration of an agreed procedure, developed by BARGE and aiming to produce a scientifically sound, robust and simple *in vitro* gastrointestinal test, as required by environmental agencies (Gron *et al.*, 2003; Environment Agency, 2007). This has been realized for metals in various matrices but there is still a need for an agreed procedure to estimate bioaccessibility of PAHs from soils using a physiologically-based extraction test.

2.4 Parameters influencing mobilization inside the gut

2.4.1 Bile salts

The mobilization of organic compounds in the gastrointestinal tract is largely influenced by the presence of bile salts (Oomen *et al.*, 2000). Generally, the main trend observed in the literature is that bile salts increase the oral bioaccessibility.

Table 2.1: Comparison of the presence of different characteristics in several *in vitro* gastro intestinal tests recently developed (Oomen *et al.*,2002)

Name of *in vitro* gastro intestinal model	Characteristics of the model					
	Static	Dynamic	Mouth compartment	Colon compartment	In vivo correlations	Food
The SBET (Simple Bioaccessibility Extraction Test); British Geological Survey, United Kingdom	X				X	
The Method E DIN 19738; Ruhr-Universitat Bochum (RUB), Germany	X				X	X
The in vitro digestion model, National institute of public health and the environment (RIVM), the Netherlands	X		X		X	X
The *SHIME (Simulator of Human Intestinal Microbial Ecosystems of Infants), LabMet (RUG)/VITO, Belgium*	X	X		X		X
TIM (TNO Gastro intestinal model); TNO nutrition, The Netherlands		X	X		X	X
Unified BARGE Method, BARGE	X		X		X	
Fed Organic Estimation Human Simulation Test , BARGE	X		X			X

A number of studies with organic compounds show clearly the influence of bile salts on the surface tension of gastrointestinal juices, the formation of micelles, and the increasing bioaccessibility values. Some work has already been done on the role of bile salts in the increase of mobilization of organic compounds in an aqueous environment (Fries, 1985). More specifically, some studies have shown increase of PAHs bioaccessibility when increasing bile salts concentrations (Hack *et al.*, 1996). Phenanthrene solubility was more than five times higher in the extracting solution containing bile salts (Pu *et al.*, 2006). Some researchers (Sips *et al.*, 2001; Wittsiepe *et al.*, 2001) observed that bile may also create an apolar environment in the interior of bile salts micelles for hydrophobic compounds. An increase with a factor 2 to 4 was reported (Hack *et al.*, 1996) in *in vitro* releases of PAHs and PCBs when their artificial digestive juices was supplemented with bile salts. Other examples such as PCBs or lindane have also shown increase in bioaccessibility when increasing bile salts amounts (Oomen *et al.*, 2000). Increased solubility of Total Petroleum Hydrocarbons (TPH) in the intestinal fluid was observed when increasing bile salts concentration, due firstly to the formation of micelles that move away from soil particles, and secondly to the decrease of the intestinal juice surface tension (Hrudey *et al.*, 1996; Holman *et al.*, 2002).

It was demonstrated that bile salts act as a surfactant or detergent, and therefore decrease the surface tension of the gastrointestinal juices substantially, which may become important for the wetting and mobilization of contaminants from soils (Laher *et al.*; Laher *et al.*, 1983; Charman *et al.*, 1997; Hack *et al.*, 1998b; Luner, 2000; Van de Wiele *et al.*, 2004). Therefore, the PAHs can be bound to the hydrophobic core of the micelles and will be more bioavailable and bioaccessible. They can be absorbed by the epithelial absorptive cells, and may enter portal blood and lymph circulation, being harmful for the human health. A description of the transport and circulation of PAH inside the gastrointestinal tract, via the ingestion of soil and food, is described in Figure 2.3.

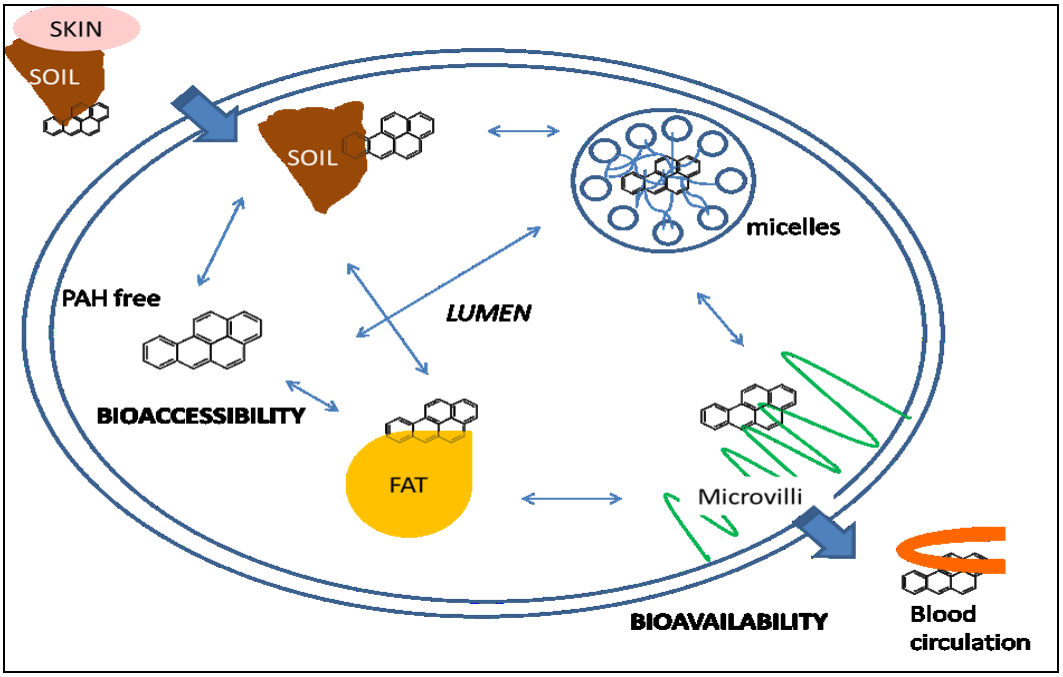

Figure 2.3: Schematic of PAH mobilization in gastrointestinal tract after ingestion of soil and food

2.4.2 Organic matter

The organic matter is known to considerably reduce the bioavailability and bioaccessibility of hydrophobic compounds in soils (Gron *et al.*, 2003). Two terms are mainly known as sequestration and weathering to define the behaviour of organic compounds within the soil organic matter (Amellal *et al.*). When a soil enters the gastrointestinal juices, the organic compounds are differently released according to the organic matter proportions. A high content in organic matter seems to decrease bioaccessibility and bioavailability as the compounds remain attracted by the soil particles, decreasing the solubility of organic molecules. Mainly because of the sorption of the contaminants on the soil organic matter which will stop mobilization of organic compounds in the gastrointestinal juices. Organic matter in soil is thought to be the most significant factor dominating organic compound interaction with soil, and thus the bioavailability of these compounds (Calvet, 1989). Other studies have confirmed that hydrophobic molecules sorption into soils was strongly dependant on organic matter (Chiou *et al.*, 1979; Karickhoff *et al.*, 1979; Chiou *et al.*, 1998)

Moreover, the hydrophobicity of a compound will favour the sequestration into the soil organic matter (Schwarzenbach *et al.*, 2003), especially important in the study of PAHs. Indeed, PAHs being hydrophobic molecules, the sequestration is again more pronounced (Means *et al.*, 1980; Chiou *et al.*, 1986; Yin *et al.*, 1996; Kogel-Knabner *et al.*, 2000; Xing, 2001; Pu *et al.*, 2004). This was also observed in the case of PCBs, PCDDs (polychlorinated dibenzo-p-dioxins) or other organic compounds where sorption and persistence was increased with an high organic carbon content (Papa-Perez *et al.*, 1991; Luthy *et al.*, 1997; Ayris *et al.*, 1999; Boehm *et al.*, 2000; Fava *et al.*, 2002).

2.4.3 Food

The main effect of adding food as part of an *in vitro* gastrointestinal model is to increase the bioavailability and bioaccessibility of hydrophobic compounds (Fries *et al.*, 1989; Hack *et al.*, 1996; Van Schooten *et al.*, 1997; Shargel *et al.*, 1999; Roos *et al.*, 2000; Wittsiepe *et al.*, 2001; Pu *et al.*, 2004). Different types of food have been added in *in vitro* gastrointestinal tests such as milk, milk powder, minced beef and grape seed oil (Hack *et al.*, 1996), with an enhancement of bioavailability, varying depending on the amount of fat contained in food. Because of the creation of apolar and lipophilic environments, as with bile salts, food is increasing solubilisation of organic and hydrophobic contaminants in the gastrointestinal juices and therefore their mobilization. Food induced mixed intestinal lipids, such as monolein and long-chain fatty acids, enhance gastrointestinal solubilisation of TPH residues (Van Schooten *et al.*, 1997; Roos *et al.*, 2000).

2.4.4 Other parameters

Some few studies have demonstrated the effect of the liquid-to-soil ratio on bioaccessibility. An increase in the liquid-to-soil ratio was showing an increase in bioaccessibility (Van de Wiele *et al.*, 2004) . They concluded that the possible effect was due to variations in dissolved organic matter. Even for a very low contaminated

soil, the resulting bioaccessibility was still significant (Van de Wiele *et al.*, 2004). However, other studies demonstrated contradictory trends with an increase or a decrease of bioaccessibility with an increasing dose of contaminants (Shu *et al.*, 1988; Wendling *et al.*, 1989; Pu *et al.*, 2003). The augmentation of the ring number in PAHs was showing a decrease in bioaccessibility in a recent study (Tang *et al.*, 2006). The organic carbon normalized bioaccessibility of individual PAHs in soils decreased with the increasing ring number in both gastric and small intestinal conditions, possibly due to the decrease in the water solubility and increase in partitioning coefficient (log K_{ow}) of individual PAHs by about one order per ring (Tang *et al.*, 2006). Comparison of drug absorption profiles with drug hydrophobicity and drug molecular weights have shown that absorption of hydrophobic drugs decline at larger molecular weight (Borgstrom, 1967; Kimura *et al.*, 1994).

As organic matter is controlling interaction between organic compounds and soils, other properties such as the type of soil can influence the sequestration of organic compounds. Typically, soils containing clay have shown a decrease in bioaccessibility, due to weak physical interaction inside the soil (Pu *et al.*, 2004; Petersen, 2007). Indeed, the high surface areas of clays involve more attraction between soil and hydrophobic compounds, therefore decreasing their mobilization inside the gastrointestinal tract. However, studies demonstrated no clear differences between bioavailabilities among soil types (Pu *et al.*, 2004) (Pu *et al.*, 2006). The mobilization of pollutants also depends on the physical qualities of the soil material such as particle structure, porosity, and grain size (cf. chapter 1) (Hack *et al.*, 1996).

2.4.5 pH and residence time

pH is also an important factor to consider in the use of *in vitro* gastrointestinal models. As described previously, the pH will give variable values according to each compartment of the gastrointestinal tract. In the mouth, the pH varies from 6 to 7.5. In the stomach the pH ranges from 1 to 4. And in the intestine, pH goes from 4.5 (duodenum) to 7.5 (ileum) (Intawongse *et al.*, 2006). The addition of food was shown

to increase pH values. For example, in the gastric part the pH was increasing from 3 to 7 (Versantvoort *et al.*, 2004). Concerning the influence of pH on the mobilizations of pollutants inside the gut, it appears that bioaccessibility are generally decreasing when pH increases, depending on the compounds (Oomen *et al.*, 2002). For instance lead bioaccessibility decreased with increasing gastric pH (Oomen *et al.*, 2003b). It was explained that the pH along with the ionic strength could influence the structure of bile salt micelles, increasing solubilisation of them and therefore bioaccessibility (Barnabas *et al.*, 1995a). Various digestion models have led to change residence time where the fluids are mixed together in order to mimic digestion movements. However, the variation between incubations times have not shown clear changes in the resulting bioaccessibilities (Intawongse *et al.*, 2006).

2.5 Development of a fed version of the *in vitro* gastrointestinal test

According to the different parameters influencing PAHs mobilization inside the gastrointestinal tract, it appears that food plays a major role. As described before, PAHs are easily mobilized in the fat constituents of the food components due to their hydrophobic properties, and they can be transported in the lumen and be potentially absorbed into the cell walls of the intestine, involving potential hazards for human health (Gron *et al.*, 2003). Moreover, including food in *in vitro* digestion models seems very essential for the development of realistic simulated gastrointestinal extraction procedures. Several studies have shown the dramatic increase in PAHs bioaccessible fractions when using a fed version of a physiologically-based extraction test (Hack *et al.*, 1996; Versantvoort *et al.*, 2004), which is important towards the evaluation of the risk to human health when ingesting PAHs via soils.

Several *in vitro* gastrointestinal tests involving food or food and soil have been used and developed (Rotard *et al.*, 1995; Hack *et al.*, 1996; Holman *et al.*, 2002; Versantvoort *et al.*, 2004; Oomen *et al.*, 2006) to assess bioaccessibility of several contaminants such metals or PAHs. However, there is still not a procedure that has

proven satisfactory robustness as with the Unified BARGE Method. In order to realize a common and robust approach, several members of BARGE, which have developed the UBM, have developed a fed version of the Unified BARGE Method, based principally on the work from the RIVM (Cave *et al.*, 2010). At the present stage, the method needs to be compared in different laboratories to estimate robustness, in order to be available in commercial laboratories, on a routine basis. A first evaluation has shown good performance of the analytical method and comparable results with another fed *in vitro* gastrointestinal test, the SHIME (Simulator of Human Intestinal Microbial Ecosystems of Infants) (Cave *et al.*, 2010). This new method was called FORES(h)t (Fed ORganic Estimation Human Simulation Test) by the BGS and was developed to analyse PAHs bioaccessibilities from soils (Cave *et al.*, 2010). It will be a fed version of the UBM, and more focused on the evaluation of PAHs bioaccessibilities, using a fed state of the digestion.

The development of the FORES(h)t method needed an understanding of the fundamental changes that occur when a digestion model includes food. The FORES(h)t method was based on a method developed by the RIVM, which justify those modifications (Cave *et al.*, 2010). Indeed, the RIVM has developed an *in vitro* gastrointestinal test including food and considering digestion of “average children” in the Netherlands. This new model was firstly based on the fact that an adult or a child is half of the day in a fed state and the other half on a fasted state (Oomen *et al.*, 2006). Therefore, applying a fed state in the simulation of soil ingestion will give a more realistic and “non-conservative” value of bioaccessibility (Versantvoort *et al.*, 2004). Addition of food involves many modifications to the gastrointestinal tract such as changes on the secretion of gastric, bile and pancreatic fluids, differences of gastric and intestinal motility patterns, and modifications in visceral blood and lymph flow (Versantvoort *et al.*, 2004). As the human physiology is significantly modified when eating food, the fed version of the *in vitro* gastro-intestinal test will involve numerous modifications. In the RIVM method, the food intake was based on food consumption during a meal from men and women aged 19-65 years old in the

Netherlands (Versantvoort *et al.*, 2004). The food constituent was chosen in order to comply with the mean intake of adults during a cooked meal, constituted with a known proportion of calories, proteins, carbohydrates and fat. Two infant formulas were chosen because the proportion of energy and nutrients are very close to those of a cooked meal. Oil was added with the infant formula to reach as nearly as possible the same amount of fat and calories, contained in a cooked meal. The amount of soil added in the RIVM test is based on the involuntary ingestion of 100 mg/day of soil considering the "average behaviour of a child" (cf. chapter 1). Therefore, the amount of soil added in the process was based on these previous assumptions. After several studies on the soil-to-solution ratio, the RIVM concluded that quantities of soil between 0.2 and 0.6 g were a good option because lower quantity of soils could lead to heterogeneity of the contaminant concentration in a soil and also give difficulties to detect some of the compounds at low levels (Oomen *et al.*, 2006). However, the FORES(h)t method is employed in England for the moment, therefore the mean intake of energy and nutrients was based on the daily food consumptions of a children aged 4-6 years old and living in the UK (Cave *et al.*, 2010).

The main changes that occur in human digestion when eating food are the residence times, the pH, the bile and pancreatic juices secretions, and the volume of food and digestive fluids (Versantvoort *et al.*, 2004): (a) in the mouth, the saliva secretion is increased. (b) Emptying the stomach when food is ingested can take more time than with a fasted state, whereas no differences are observed in the small intestine. (c) In the small intestine, the bile secretion is increased, until food is removed from the stomach. (d) The pancreatic secretion also increases significantly in the duodenum when food is part of the digestion. The pH gradually increases also after eating, with a pH increasing from 1.5-2 to 3-7 in the stomach and from 5.5 to 7.5 in the duodenum and ileum, contrary to the jejunum where there are no differences. (e) Finally, the volume of digested fluids will depend on the amount of food and liquid ingested during a meal, and therefore will vary between a fasted and a fed state. Based on these

assumptions the RIVM has developed a fed physiologically-based extraction test for the analysis of metals and lipophilic compounds (e.g : benzo(a)pyrene) in various matrices such as soil or food (Versantvoort *et al.*, 2004). When applying the fed version of a gastrointestinal model the scientists from the RIVM demonstrated that the food was clearly increasing the bioaccessibility (from 5 to 43 %) of benzo(a)pyrene, but not of the metals Pb, As and Cd due mainly to the lipophilic character of benzo(a)pyrene (Versantvoort *et al.*, 2004).

When developing this model, four important considerations were made, as it is in general for the development of other *in vitro* gastrointestinal tests: absorption (1) will occur in the intestine, so a model involving only the stomach is not sensible. This test (2) should represent a worst case scenario, however the model should be as realistic as possible. The scenario (3) involved will depend on the contaminant and on the matrix studied. Finally (4), the test needs to be easily applicable, robust and reproducible (Versantvoort *et al.*, 2004).

2.6 Validation of the method: quality and reproducibility

The reproducibility is an important parameter into the validation of those models. However, little differences between laboratories procedures such as the separation of the chyme from the matrix (using filtration and centrifugation), can produce variation in bioaccessibilities (Versantvoort *et al.*, 2004). These considerations need to be kept in mind when comparing bioaccessible fractions from different laboratories, including also the type of shaking, the vessels used and the purifications methods, which can lead to variables results (Versantvoort *et al.*, 2004), as uncertainty is always significant in any analytical procedures. However, reproducibility of the model with various contaminants in food or soil was shown acceptable by observing between day variation varying from 9 to 54 % (mean 25 %), within day variation varying from 3 to 74 % (mean 17%) and minor variations of pH (Versantvoort *et al.*, 2004). A recent inter-laboratory study from BARGE has also shown satisfactory reproducibility for the

application of the Unified Barge Method on metals within soil matrices (Wragg *et al.*, 2009). The next step would be to establish the robustness of the FORES(h)t method, as the method has now been implemented and validated in one laboratory (Cave *et al.*, 2010). However, to realize these inter-laboratory studies, the RIVM suggest the use and preparation of reference soil samples in all various institutes to allow a quality control, as bioaccessibility could vary from one laboratory to another (Oomen *et al.*, 2006). As there are no actual reference materials to use in bioaccessibility studies (for PAHs), this will provide a "uniformity" of the results between laboratories (Oomen *et al.*, 2006).

2.7 Consideration in risk assessment

The final aim of introducing a fed state of a simulated gastrointestinal model, as with a fasted state, is to refine the risk assessment from pollutants in various matrices (Environment Agency, 2005). Especially for PAHs, the fed state is an important step forward, because it describes more clearly the mobilization of hydrophobic compounds inside the gastro-intestinal tract. Indeed, interactions will occur with the fat components and the bile salt micelles inside the lumen, favouring the absorption into the layers (microvilli) of the small intestine (Gron *et al.*, 2003).

Comparing the bioaccessibility of compounds from different matrices, the RIVM concluded that the bioaccessible fraction was variable according to the matrix studied, and also that not all the pollutants were released from the matrix in the same manner (Oomen *et al.*, 2006). The bioaccessible fraction should be reported as the maximum fraction of a contaminant that could be available from the human body, thus improving the risk assessment (Versantvoort *et al.*, 2004).

2.8 Extractions methods and analysis following PBETs

After realizing an *in vitro* gastrointestinal procedure, the final solution can contain a complex mix of components such as biological fluids, soil particles, and food that can seriously harm the instruments used for further analysis. Secondly, targeted

compounds need to be isolated and clearly identified, in order to obtain final concentrations. Typically, the extraction procedures for liquid matrices developed and used in the recent decades are, liquid-liquid extraction, solid-phase extraction, solid phase micro-extraction, stir-bar sorptive extraction and micro-extraction by packed sorbent.

Liquid-liquid extraction is the most common method, and consists in mixing two solvents with different properties in order to isolate a compound which has an affinity with one of the two phases.With solid phase extraction, the sorbent permits to retain the compounds, and to get them in lower quantities of solvent. This technique allows purification and pre-concentration of samples. To summarize the process (Figure 2.4), the first step consists of conditioning the sorbent by adding a specific solvent. This will swell and expand the phase. The long grafts (C8 or C18 for example) will be straightened in order to permit interactions. Indeed, the C18 octadecyl-bonded silica network will be spread and more available to non-polar compounds. Then, they will be more attractive to molecules reaching the sorbent with the same properties.

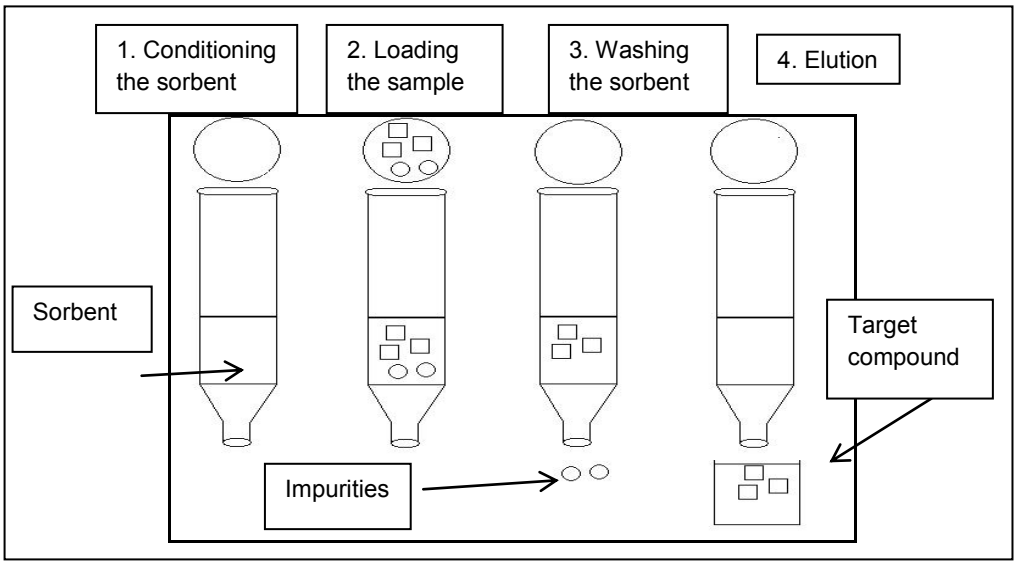

Figure 2.4: Schematic of the Solid-Phase Extraction procedure

The second step washes the sorbent with solvent. The third step consists of loading the sample (1 L or less) in the cartridge passing through the sorbent. And the final step permits to elute the compounds remaining on the sorbent with a small quantity of

solvent, being soluble with the compounds. The lower chain C8 and C2 retain less the PAHs because of the chain length which attract less. C2 is more polar due to the exposition of the polar group (Si-O). Indeed, the short C2 alkyl group provides a smaller area for non-polar interaction to occur. Therefore, C18 will be really appropriate for the extraction of PAHs due to its high hydrophobicity compared to other sorbent such as C8 or C2 which are more polar, therefore less attractive to PAHs.

Stir Bar Sorptive Extraction is a sample preparation technique that involves the extraction and enrichment of organic compounds from a liquid sample, using a stir bar which is covered with a coating, for example PDMS (Figure 2.5). The extraction time is controlled kinetically. It is determined by the sample volume, the stirring speed and the stir bar dimensions and must be optimized for a given application (Kawaguchi *et al.*, 2005).]

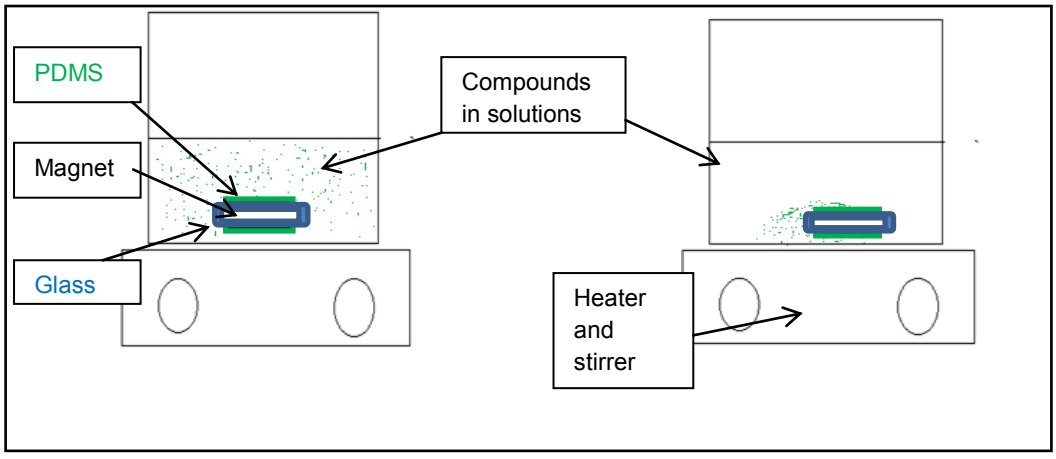

Figure 2.5: Schematic of a Stir-Bar Sorptive Extraction principle with PDMS coating (Barnabas *et al.*, 1995b)

To realize desorption of the compounds from the stir bar, either thermal desorption or liquid desorption can be used. The former includes a thermal desorption unit fixed on the instrument (GC-MS). Then, the stir bar is directly placed in the unit and molecules are desorbed at high temperature. The latter consist to heat and sonicate a small quantity of liquid with the stir bar to desorb the compounds. The PDMS is very

suitable for the analysis of PAHs because it is an apolar stationary phase and therefore it is very attractive for non-polar compounds (Figure 2.6).

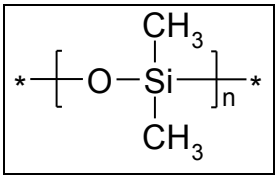

Figure 2.6: PolyDiMethylSiloxane (PDMS) molecular structure used with SPME and SBSE

Solid-Phase Micro-Extraction is an equilibrium extraction technique and a solvent-free method (Pawliszyn, 1999). The fibre has a polymer coating chosen for its suitability for the analytes of interest. For example, for relatively non-polar compounds such as PAHs, a non-polar coating such as polydimethylsiloxane (PDMS) is used, as with the SBSE technique (Figure 2.6 an 2.7). The thickness of the coating can also be varied between 7 and 100 µm, thin coatings are generally most effective for the adsorption of semi volatile analytes, while thicker coatings should be used for volatile compounds (Supelco, 1998).The fibres are either immersed in the sample or exposed to the headspace above it. Analytes in aqueous samples can be extracted by immersion (King *et al.*, 2003). The PDMS fibre attracts the compounds when in stirring solution in a specific solvent. Then, the fibre is inserted in the injection port of the GC-MS for desorption of the compounds at high temperature.

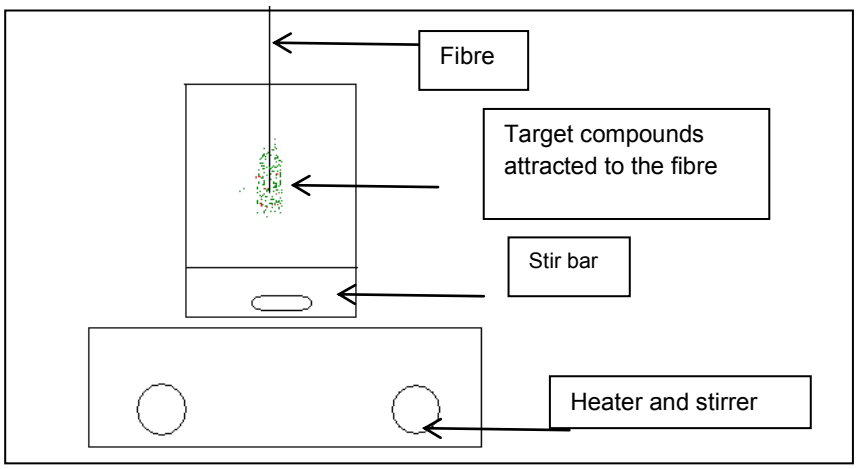

Figure 2.7: Schematic of the Solid-Phase Micro-Extraction principle

Micro Extraction by Packed Sorbent is a new and interesting technique of which the concept is similar to SPE, and the main advantage is to reduce the sample extraction time. This tool can be directly connected to a GC or LC during operation (El-Beqqal *et al.*, 2006) Indeed, in this device, the sorbent (BIN) is fixed inside a 250 ml syringe. The sorbent material or solid packing material used in the packed syringe can be silica-C8, silica-C18 or any type of sorbents (Figure 2.8). This method requires the same four steps as with SPE, but all the process can be done with a small amount of sample (10 ml or less): Conditioning the sorbent, sampling multiple times, washing, and eluting directly in the GC-MS with a large amount of solvent (50 µl). The multiple pulling and pushing of the sample by the syringe increases the extraction recovery (El-Beqqal *et al.*, 2006). MEPS can be employed with Programmed Temperature Vaporizing injector (PTV) with GC-MS which allows large volume of injection compared to Split/Split less mode (SSL). With the PTV injector, the sample is deposited into the inlet at a low temperature, and then the inlet temperature is rapidly raised to vaporize the desired compounds and cause their transfer to the column. The PTV can operate effectively with large volume injections allowing venting of solvent and backflushing of undesired compounds to vent (Clay *et al.*, 2004) PTV/LVI is used mainly to increase sensitivity and to deal with complex samples containing impurities.

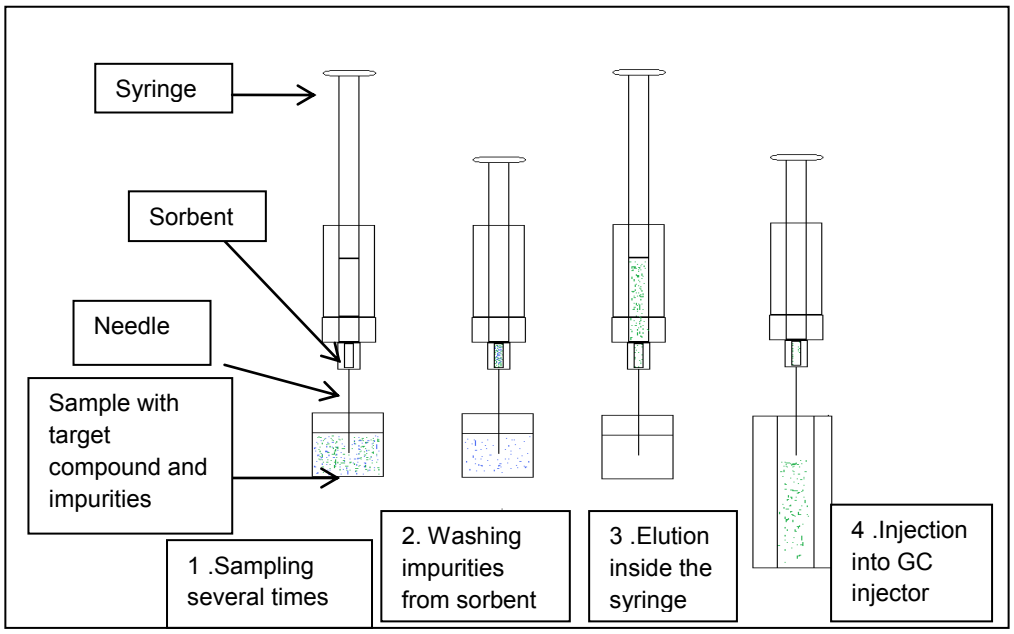

Figure 2.8: Description of the Micro-Extraction by Packed Sorbent

2.9 Conclusion

The *in vitro* gastrointestinal models are complex tools that are trying to simulate human digestion, in order to consider the effect of contaminants after ingestion of environmental matrices. To estimate this effect, the concentration of the contaminant is measured at the end of the process, after using appropriate analytical methods to isolate the target compounds. This concentration is divided by the total content in the matrix and is called bioaccessibility. The bioaccessibility is an indicator of bioavailability, but a contaminant that is bioaccessible is not necessarily bioavailable because of the complex mechanisms that are involved for the transfer of a compound from the lumen to the systemic circulation (Gron *et al.*, 2003). Therefore, *in vivo* evaluations are always important to validate *in vitro* studies, on a particular contaminant present in a specific matrix. Currently, various *in vitro* gastrointestinal models have been implemented and validated in certain laboratories (Oomen *et al.*, 2002). BARGE (BioAccessibility Research Group of Europe) is an international group of researchers who are trying to compare and evaluate those models, in order to establish a common and accepted physiologically-based extraction test for an international standardization of the use of bioaccessibility (Hansen *et al.*, 2007). They have developed a physiologically-based extraction test called the Unified BARGE Method which has shown good performance in terms of robustness. This model has been applied for numerous studies, such as metals in environmental matrices (Cave *et al.*, 2006). Furthermore, it has been demonstrated that an important number of parameters are influencing the mobilization of contaminants inside the gastrointestinal tract, such as food, bile salts, mucine, pH and soil properties. Therefore, other types of simulated digestion models are being developed in order to be more realistic. The last model being developed by the BARGE is based on the Unified BARGE Method, but is a fed version. It is based on some previous work from the RIVM on the addition of food matrices in the *in vitro* gastrointestinal tests (Oomen *et al.*, 2006). This model is particularly adapted for environmental matrices contaminated with polycyclic aromatic hydrocarbons. Those toxic compounds are very hydrophobic, so they are

easily retained by the fat constituents of the food, and also into the core of the bile salt micelles, leading to more absorption into the epithelial cells covering the inner layer of the small intestine. This test will permit to avoid underestimation or overestimation of the risk caused by the ingestion of PAHs via environmental matrices. The fed model being developed is called the FORES(h)t (Fed ORganic Estimation Human Simulation Test) (Cave *et al.*, 2010). At this stage, the method still needs to be further validated through interlaboratory studies, and the researchers need to agree on one type of reference material that all laboratories could use, in order to get a uniformity of the results, replacing the use of a certified reference material, which is not available for PAHs in bioaccessibility studies (Oomen *et al.*, 2006).

The use of *in vitro* models is therefore essential into the refinement of the human health risk assessment. Firstly, because the simple use of a physiologically-based extraction test will give access to the bioaccessible fraction, which will give more information about the risk than the comparison with soil guidelines values based on possible biased assumptions, using various matrices (Environment Agency, 2005). Secondly, by combining the use of that model with food, the estimation of PAHs bioaccessibility *via* ingestion of solid environmental matrices, will be again more accurate. However, it needs to keep in mind that even if bioaccessibility can be a powerful tool to estimate the human health risk, *in vivo* correlations and inter-laboratory studies need to be considered.

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Chapter 3: Sampling, preparation and analysis of contaminated urban soils

3.1 Introduction

The sampling and analysis of environmental matrices need to be planned and meticulously organised in order to obtain pollutant levels with as little uncertainty as possible. These procedures are required to let public and environmental scientists know about the risk of living and having activities close to contaminated areas, in the case of soil guidelines values exceeded, for example. In Newcastle-upon-Tyne, an old Tar works site has contributed to the contamination of the environment and the land close to the Tyne River. People have activities (fishing, walking) and are living very close to the area, potentially involving risk for their health, as they can be in contact with the soils (Newcastle City Council and Ove Arup and Partners, 2007). The Newcastle City Council has therefore installed warning signs to prevent people accessing the site, as a first step towards prevention of the risk in the area. However, further action will be needed to confirm there is a risk, using appropriate practical methods and complex tools for (1) sampling, (2) pre-treatment and storage, (3) extraction and (4) analysis (Dean, 2009).

The sampling strategies really depend on the objectives of the study and are generally separated in several types such as random, stratified and judgemental (Keith, 1991). After being collected, samples cannot be extracted and analysed directly, so they should be stored in appropriate containers (Keith, 1991). The type of storage and pre-treatment method will need to consider the reactivity of the analyte with light, air, heat, water, biological organisms, metals and other reagents. Moreover, parameters such as compound volatility, space and time variability, and compound sorption to the sampling tool and container will need to be considered when storing samples (Keith, 1991). The next step after storage is to choose the appropriate method to realize the extraction of compounds from the matrix. This step will follow strict quality requirements in order to notice any errors that will be controlled after with instrument

analysis. Generally, the quality control will require the analysis of spikes, blanks, duplicates and certified reference materials.

To analyse pollutants in solid environmental matrices there is a large number of extractions methods existing nowadays. The current EPA methods for this usage are: Soxhlet extraction (Method 3541, 1994), automated Soxhlet extraction (Method 3540C, 1996), pressurize fluid extraction (PFE) (Method 3545, 1996), ultrasonic extraction (Method 3550C, 2007) and supercritical fluid extraction (SFE) (Method 3561, 1996). Recently, PFE was shown to be a more effective way to extract PAHs from soils compared to other extraction methods (Bjorklund *et al.*, 1998; Dean, 1998) in terms of accuracy, precision, reduced time of operation and reduced use of solvent.

After extraction, the solution containing the targeted compounds is almost ready to be injected in an appropriate analytical instrument. However, before injection, some considerations must be made on the necessity to use clean-up procedures to eliminate impurities and incompatible reagents that could harm particular compartments of the analytical instrument. Generally, clean-ups methods involve the utilization of a sorbent which will remove the unwanted impurities or compounds. The sorbent can be placed in a suitable chromatographic (glass) column with a drying agent to realize the clean-up procedure. Recently, clean-up methods have been integrated in extraction procedures such as PFE or SFE (Fidalgo-Used *et al.*, 2007), they combine the extraction and the purification in only one step, which reduce time of manipulation and costs. Finally, concerning the analysis of polycyclic aromatic hydrocarbons in environmental matrices, the type of instrument used is generally GC-MS and HPLC-UV (or fluorescence), but GC-MS methods are generally more employed because of better selectivity, resolution and sensitivity (Poster *et al.*, 2006).

The purpose of this chapter is to briefly present (1) the sampling strategy for soil and dust samples, (2) the sample pre-treatment and storage of such matrices, (3) the extraction and purification procedures available for these matrices, and finally (4) the

different types of instrument analysis for PAHs in soils and dust, which already exist in the literature.

3.2 Sampling strategy

It is important to keep in mind that in any analytical processes, which involve numerous steps such as sampling, pre-treatment procedures, instrument analysis and data treatment, uncertainty of measurement is always present. The error estimated on each step has been reported (Markert, 1995) and involves up to 1000 % for representative sampling, 100-300 % for sample preparation, 2-20 % for instrument measurement, and finally 50 % for data treatment (Markert, 1995; Theocharopoulos *et al.*, 2001). Therefore, potential error on the sampling strategy and pre-treatment procedures is significantly higher than for the extraction, analysis and data interpretation (Fortunati *et al.*, 1994). This fact shows the need to prepare an analytical procedure on a precise and strategic sampling approach. Indeed, the sampling strategy is the first and indispensable step in the process of isolating pollutants from environmental matrices. In the case of solid matrices such as soils and road dust, the site needs to be thoroughly explored before starting to go for sampling, and choices have to be made on the type of sampling that will be made on the site, according to the type of data analysis realized afterwards (Dean, 1998). Generally, background information and historic ordnance survey maps about the sampling site should be obtained from the city council, which will include a general description of the location, site history, and more detailed information about surface geology, stream flows, river locations, archaeological issues and any possible former quarrying activities (Dean, 2009). Moreover, a site walkover should allow the investigators to notice main features of the site such as houses, roads, people activities, in order to evaluate potential risk for humans (Dean, 2009).

The information collected from the historic ordnance survey maps can give precious clues about the future distribution of contaminants on the site, such as a particular building involved in the production of chemicals, which could have polluted the

environment (Dean, 2009). When the necessary background knowledge about the site has been acquired, the investigators can decide what type of sampling approach they will use and the material required. The sampling approach will consider the selection of the sample points, the size and the shape of the sample area, and the number of sampling units in each sample (Dean, 2009). Those processes will vary according to the purposes of the study and the type of sampling site (Keith, 1991). The three types of approaches are judgemental, systematic and random. These methods can be combined into other forms of approaches such as judgemental random, systematic random and systematic judgemental. Considering the systematic sampling, the samples can be taken randomly inside the grids drawn for the sampling. All methods have advantages and drawbacks and are listed in Table 3.1. Sometimes, methods can be mixed according to the type of site and the sample locations (Keith, 1991). In all cases an appropriate decision for the sampling approach should be made, taking account of the possible distribution of the contaminants on the site.

Table 3.1: Primary sampling approaches (Keith, 1991)

Approach	Relative Number of Samples	Relative Bias	Basis of Selecting Sampling Sites
Judgemental	Smallest	Largest	Prior history, visual assessment of technical judgement
Systematic	Larger	Smaller	Consistent grid or pattern
Random	Largest	Smallest	Simple random selection

When the preliminary decisions on the sampling strategy are made, investigators need to consider appropriate clothes, protection, sampling tools (trowel, “Kraft” bags) and cleaning devices (to avoid cross contamination) when going on the site. Separate devices for each sample can, for instance, be used before doing the decontamination later in the laboratory (Keith, 1991). Considering the collection of road dust samples, either sweeping tools (Yang *et al.*, 1997; Hassanien *et al.*, 2008; Duran *et al.*, 2009), or vacuum cleaners (Yang *et al.*, 1995; Dong *et al.*, 2007; Zhao *et al.*, 2009) were used.

3.3 Pre-treatment and storage of samples

After collection of the samples from the site, samples containing PAHs need to be treated in order to start the extraction procedure. It would be ideal to start directly the extraction after collection, but the amount of samples and the sample condition often require a pre-treatment procedure (Keith, 1991). Indeed, after collection, samples can be wet, and can be mixed with rocks and debris. Therefore they need to be dried before extraction, and also to be sieved at a certain particle size owing to the type of information required from the analysis. In a large number of studies involving ingestion of soils, samples were sieved at a particle size below 250 µm, considering the potential adsorption of that specific soil particle size into children's fingers via hand-to-mouth behaviour (Bornschein *et al.*, 1987; Rodriguez *et al.*, 1999; US Environmental Protection Agency, 2000). Generally, soils are firstly sieved under the particle size of 2 mm to remove stones, large roots and other materials (Dean, 2009), before going down to lower grain sizes, according to the pathway of exposure studied (inhalation, ingestion). There are different ways of realizing the drying of the soil samples which can be also applied on dust sample, which generally involves less exhaustive pre-treatment procedure, requiring only the drying and sieving steps (Yang *et al.*, 1995; Dong *et al.*, 2007)**.** Thermal drying seems to be the most frequently used method (Berset *et al.*, 1999). However, this technique can involve loss of analytes if the temperature of drying is taken too high. Apparent losses started to appear above 40 °C for PAHs (Berset *et al.*, 1999). Probably the lower molecular weights PAHs, such as naphthalene, were lost because of their volatile character. Researchers concluded that an appropriate temperature between 25 °C and 40 °C to dry the soil could be suitable to avoid losses. Air-drying of the soil in a fume cupboard could be also a solution, bearing in mind that letting a sample exposed to the laboratory air can involve contaminations (Keith, 1991). Another way is to freeze-dry the samples, and it has become a commonly used storage technique in the recent years (Berset *et al.*, 1999). It is particularly adapted for non-polar compounds such as PAHs, and it is more rapid compared to thermal drying. Moreover, this method permits to avoid

possible air contamination that could occur through air-drying. However, in one study it appeared that naphthalene was lost using this freeze-drying procedure (Berset *et al.*, 1999) or when stored immediately after collection in a freezer (Krauss *et al.*, 2003). Air-drying was generally used for the analysis of PAHs in soils (Wilcke, 2007), or in some cases soils samples were field-fresh extracted (Chu *et al.*, 2003), or freeze-dried (Berset *et al.*, 1995). A comparison between air-dried and field-freshed extracted samples (frozen directly after collection) showed that a consequent percentage of PAHs were lost during the air-drying procedure, which was attributed to the volatility of low molecular weight PAHs and the sequestration of compounds on the soils with time (Wilcke, 2007). When those drying methods are not convenient, chemical drying could be chosen. In this case, the soil sample is mixed with anhydrous sodium sulphate and talcum powder cooled in liquid nitrogen and milled (Berset *et al.*, 1999). This drying method has several advantages compared to the other techniques presented previously: (1) water is trapped via binding to sodium sulphate, (2) soil is not exposed to laboratory air for a long time, (3) the microbial activity is stopped due to the abundant presence of salt and (4) samples could remain longer in the freezer without risk of degradation. However, the addition of salt into the solution provokes dilution of the sample. Naphthalene appeared with a higher concentration (or recovery) using this technique compared with other drying methods (Berset *et al.*, 1999). This specific study comparing different drying methods showed that except the variability of results for naphthalene, the rest of the 16 PAHs were situated the same range of values, demonstrating that the choice of the drying procedure will not lead to significant modifications in the final results.

After drying of the soil samples during a certain time, the samples needs to undergo homogenization (mixing/crushing), grinding (mills, mortar) and sieving, in most of the cases before storage and extraction (Okorie, 2010). During those stages, the tool used must not be interacting with the contaminants requiring analysis in the matrix. Contamination can appear due to the type of container and the tools employed. Then,

contamination problems can be avoided by using appropriate equipment and having good laboratory practices (Okorie, 2010). For example, when working with hydrophobic compounds such as PAHs, it is better to avoid the use of plastic as it can retain these types of molecules. The conditions of storage requires the same type of precautions, generally the glass containers are preferred, with a polyethylene or poly (tetrafluoroethylene) lid, and it is advised to avoid letting a headspace above the sample, as it can involve oxidation reactions (Dean, 1998). Contaminations can also occur by abrasion of the mortar and pestle with a sample matrix when grinding (Okorie, 2010). The next step of the analytical process for the analysis of pollutants in solid environmental matrices will require other considerations. Uncertainty is still present during extractions, but, in this step, the cost of the instrument and reagents used, the rapidity of the extraction, and the consistency and accuracy of results, will be more important.

3.4 Extraction and purification procedures

3.4.1 Extraction of PAHs from solid environmental matrices

To realize the extraction of PAHs from solid environmental matrices, the method used generally were soxhlet extraction, alkaline saponification, ultrasonic extraction and accelerated solvent extraction (Wilcke, 2007). Investigation of several types of extraction procedures is usually done to reduce the time of operation and the use of large amount of solvent. The most commonly used technique for the extraction of PAHs from soil samples is soxhlet extraction, other techniques more recently applied are soxthern, supercritical fluid extraction and microwave extraction (Graham *et al.*, 2006). Shaking methods and ultrasonic extraction were applied since a long time but were considered to be less effective than the other type of extraction methods (Jonker *et al.*, 2002). Comparing the use of soxhlet extraction, microwave-assisted extraction and pressurized fluid extraction, one study demonstrated that the best repeatability and recoveries were obtained with PFE (Pressurized Fluid Extraction) in the first position, then MAE (Microwave Assisted Extraction) and finally Soxhlet (Itoh *et al.*,

2008). This was confirmed by a similar study comparing the three methods and showing the best extraction efficiency for PFE compared to MAE and soxhlet (Wang *et al.*, 2007). It was concluded that PFE and MAE were more suitable methods for the extraction of PAHs from soils. SFE was also shown to be a good technique to extract PAHs from soils compared to methods like soxhlet, ultrasonic and shaking. Methanolic saponification demonstrated very good PAHs recoveries but variations were observed for the low molecular weights PAHs (Berset *et al.*, 1999).

3.4.2 Pressurized fluid extraction

The principal components of a PFE system are a source of organic solvent, a pump to circulate the solvent, a sample cell into which is placed the sample, an oven in which the sample cell is heated and its set temperature monitored, a serie of valves that allows pressure to be measured and generated within the sample cell, and an outlet point (Figure 3.1) (Dean, 2009). This method allows reaching very high temperature of extraction without evaporating the solvent. This is explained by the fact that pressure is applied on the cell where the sample is placed, so when the solvent is mixed with the sample, temperature above boiling points can be reached, improving the extraction efficiency (Fidalgo-Used et al., 2007).

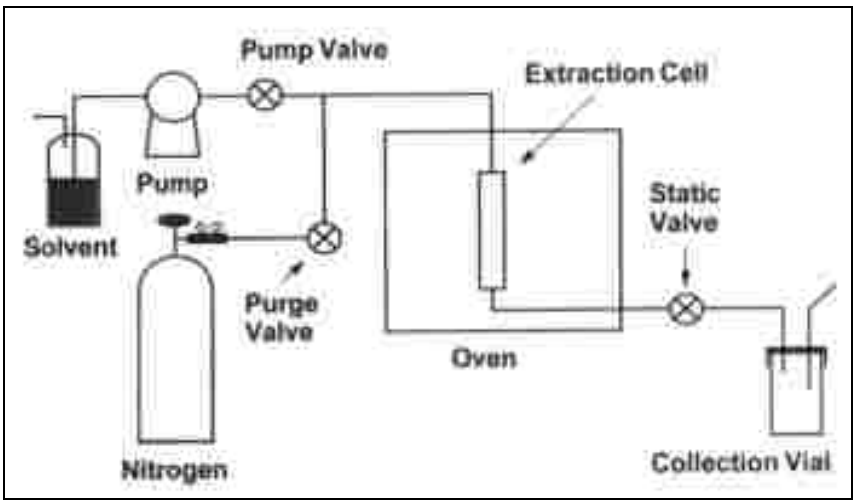

Figure 3.1: Schematic of the principle of Pressurized Fluid Extraction (Cyberlipidcenter, website)

3.4.3 Clean-up methods

Generally, column chromatography or solid phase extraction were used with florisil, silica gel, aluminium oxide, or a combination of them, in different mesh sizes, with various types of solvents to elute PAHs resulting from the extraction of solid environmental matrices (Wilcke, 2007). Other types of sorbents, or tools such as filtration, are obviously available and depend on the type and structure of the compounds studied and the interference that needs to be removed (oil, food, etc), and also on the matrix. The US Environmental Protection Agency has approved a list of protocols for the application of the clean-up with the same type of sorbent such as alumina, florisil, and silica gel for the purification of organic extracts from solid environmental matrices (Method 3610B, 1996; Method 3620 B, 1996; Method 3630 C, 1996; Method 3660, 1996). Generally, each of the method considers the clean-up using column chromatography or solid phase extraction. The former uses larger amounts of solvent and adsorbent but has a greater clean-up capacity (Method 3620 B, 1996).

A new type of clean-up that is being used for various solid environmental and non-environmental matrices combine the extraction and the purification in only one simultaneous step and is called on-line, in cell (for PFE), *in-situ* or selective clean-up (Gomez-Ariza *et al.*, 2002; Bjorklund *et al.*, 2006; Fidalgo-Used *et al.*, 2007; Hussen *et al.*, 2007; Westbom *et al.*, 2008). It consists in realizing the clean-up simultaneously with the extraction, by adding the sorbent inside the cell, in the case of PFE. Few publications have been dealing with this integrated form of clean-up and generally involve the PFE and SFE techniques (Fidalgo-Used *et al.*, 2007). The objective of this one-step clean-up is to reduce (1) extraction and purification procedure time, (2) cost, and (3) use of large volume of solvent. The method has shown efficiency for the extraction and purification of PAHs from soil samples (Kim *et al.*, 2003). The difference in recoveries between the off-line and on-line clean-up, in this study, was not significant (less than 5%), and the extract was very clear and contained less

impurities using the latter method (Kim *et al.*, 2003) The comparison of the on-line and off-line procedure shows clearly that the former method is faster, easily automated and consumes less solvents (Gomez-Ariza *et al.*, 2002; Fidalgo-Used *et al.*, 2007).

3.4.4 Clean-up using copper powder

According to the literature, copper is used to reduce the sulphur content in soils (Berset *et al.*, 1999; Notar *et al.*, 2000). This clean-up is realized because high sulphur content in soil can involve bleeding and deterioration of the GC-MS column. Moreover, recently, the copper powder has been used directly into the cell of the PFE, as described before for the clean-up with sorbents such as florisil or alumina (Notar *et al.*, 2000). However, one study demonstrated no effect on the sulphur removal with copper addition using a selective PFE (Rodil *et al.*, 2008).

3.4.5 Pre-concentration step

When finalizing the preparation of the samples for injection in the instrument, pre-concentrations of the solutions can be done in order to improve signal sensitivity. The most common approach of pre-concentration step are gas blow down, Kuderna-Danish evaporative concentration, the automated evaporative concentration system (EVACS), or rotary evaporation (Dean, 2009). Care should be taken when doing the evaporation, firstly because compounds can be lost due to their high volatility or low ebullition point, and secondly contamination due to the glassware can appear. Sometimes solutions can be evaporated directly to dryness and reconstituted with a small known quantity of solvent before injection into the analytical instrument.

3.5 Analysis

3.5.1 Description of gas chromatography-mass spectrometry principle

Gas chromatography is used to separate components in a liquid or gaseous phase according to their respective polarities and ebullition temperatures, via a mobile phase which is the gas and a stationary phase which is the capillary column. Generally, a

small amount of liquid (1-5 µl) is injected via a heated injection port, through a rubber septum, and the sample is volatilized before entering the column (Figure 3.2). The most commonly used injector is the split/splitless mode, where a certain amount of sample is more or less vented before injection. To authorize a higher quantity of sample (30-50 µl) to be injected, the PTV (programmed temperature vaporizing) can be used. The injection can be done manually or using an autosampler. Then, the sample is transferred into the column inside the GC oven (Figure 3.2), which is generally made of polyimide-coated silica with dimensions of between 10 and 60 m, long with an internal diameter between 0.1 and 0.5 mm, and a cross-linked silicone polymer stationary phase (for instance 5 % polydiphenyl-95 % polydimethylsiloxane, coated as a thin film on the inner wall of the fused silica (SiO_2) capillary of thickness 0.1-0.5 µm (Dean, 2009). The compounds will be separated into the column according to a temperature program, established for the oven.

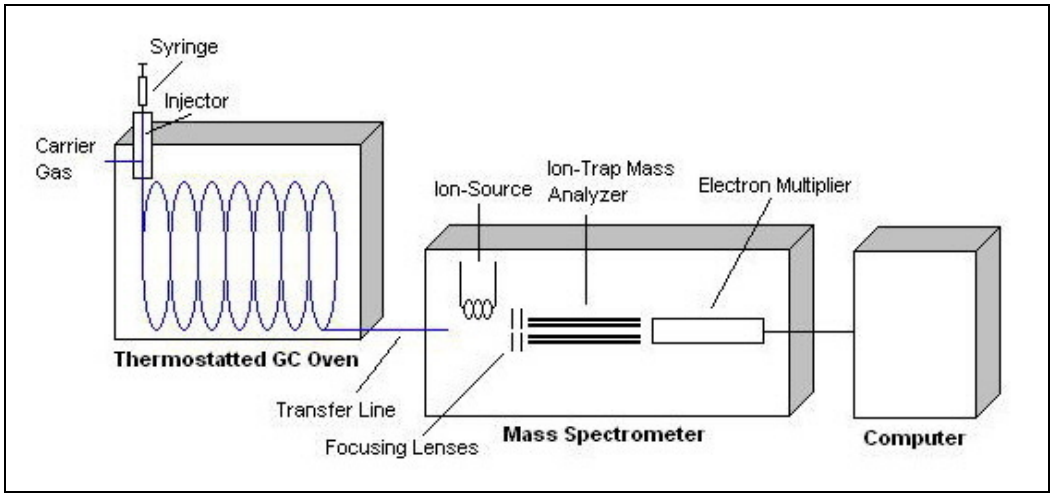

Figure 3.2: Principle of Gas Chromatography-Mass Spectrometry (UCDavis Chemwiki, website)

Then, detection can occur using a mass spectrometer or a detector such as Flame ionization detector (FID), Electron Capture Detector (ECD), Photo-ionization detector (PID), Flame photometric detector (FPD) , NPD (nitrogen-phosphorous detector), and thermal conductivity detector (TCD). The type will be chosen according to the type of compounds that are analysed, such as organic compounds, phosphorous or

nitrogenous compounds, halogenous compounds, metals and aromatic compounds, etc. The most universally used detector is the FID and the mass spectrometer (such as quadrupole, ion trap or time of flight). When using a mass spectrometer as GC detector, the compounds are exiting the column from the transfer line and are bombarded by electrons produced by a rhenium filament. This phenomenon is called electron impact and produces charged species (ions) which can be separated according to their mass to charge ratio (m/z) into the mass spectrometer. The ions are then entering an electron multiplier which will produce electrons by collision, finally producing a signal response which is proportional to the amount of organic compound. The data will be finally collected either in full scan which means that all ions will be detected, or in single (selected) ion monitoring where only selected ions will be recorded.

3.5.2 Analysis of PAHs using chromatography and mass spectrometry

The main drawback for the analysis of PAHs in environmental matrices is the difficulty to separate isomers. This can be overcome by using other types of detector such as various mass spectrometers or the Flame Ionization Detector. The main types of instrumental analysis for PAHs in soils involve the use of a GC-quadrupole, GC-ion trap (Nam *et al.*, 2003) or GC-FID (Wilcke, 2007). However, PAHs analyses by GC-TOF, GC-IRMS, LC-MS and HPLC have also been reported (Poster *et al.*, 2006). Generally, the analysis of PAHs by capillary gas chromatography involved the utilization of methyl and phenyl substituted polysiloxanes columns due to their low polarity which will permit retention of hydrophobic compounds. The type of column used can influence the separation of PAHs, the signal sensitivity, the resolution and the selectivity (Poster *et al.*, 2006).

3.6 Quality assurance and quality control

In order to control the quality of data obtained in a specific laboratory with a specific user, some specific points should be addressed. Generally, the use of certified

reference materials in extractions is recommended to compare values obtained by a single laboratory with certificate values. Samples should be at least extracted and analysed three times so as to observe precision and accuracy of the results. The same should be done with spiked samples or blanks where recoveries will be observed in order to evaluate again the performance of a specific method. A calibration with 5 to 7 standards must be realized before starting analysing samples, and must show correlation coefficients above 0.995 (for all PAHs), demonstrating good linearity of the prepared standards for future quantification of pollutants in samples. The stability of the calibration should be checked every day by controlling the response of a standard used in the initial calibration. It could also be useful to run a calibration and compare the response after 24 hours or 48 hours to evaluate the stability of the standards. Finally, blanks should be analysed to control the presence of compounds between batches, and permit to check the purity of the solvent, avoiding errors and increased uncertainty with results (Dean, 2009).

3.7 Conclusions and aims of the project

Based on this entire approach the samples from this study will need to be collected strategically, stored and treated appropriately avoiding losses, extracted using a simplified, rapid, accurate, precise, robust, realistic and non-expensive approach, injected using a sensitive approach allowing good separation, following strict quality guidelines, in order to get the lowest uncertainty in the final results.

The main objectives of this thesis are presented below and are summarized on Figure 3.3:

(a) To develop an appropriate and efficient analytical method to isolate PAHs from any solid environmental matrices based on an *in-situ* PFE approach (Chapter 4).

(b) Apply this method on soil matrices from a former contaminated industrial site in order to determine total PAH content, consequently the risk on the site, and compare

distribution according to two different particle sizes, considering the ingestion exposure pathway (Chapter 5).

(c) Implement and compare fasted and fed *in vitro* gastrointestinal tests in the present laboratory coupled with analytical methods, evaluating PAHs bioaccessible fractions and the human health risk, and evaluate the robustness of the method using an inter-laboratory study (Chapter 6).

(d) To apply this method on road dust in order to assess the risk by evaluating the potential daily intake of PAHs via involuntary ingestion of dust particles, considering again various particles sizes, and identify the various sources of PAHs in road dust. (Chapter 7)

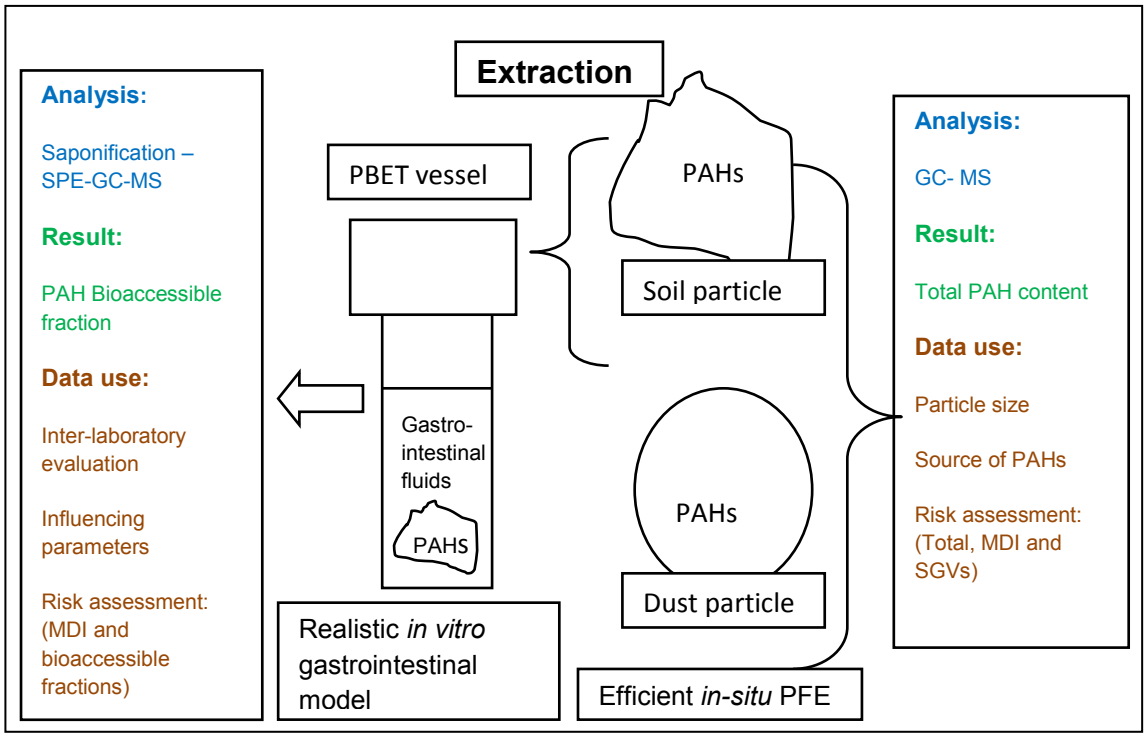

Figure 3.3: Schematic representation of the aims of the thesis

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Chapter 4: Development of a method to establish total PAH content in soils

4.1 Introduction

In order to evaluate the risk from the occurrence of pollutants in a particular matrix, appropriate extractions, purifications and analysis methods must be used and developed. Chromatographs and mass spectrometers are very appropriate for the analysis and quantification of a large range of analytes. However, before being analysed, the compounds need to be isolated from the matrix using suitable extractions and purifications, by employing specific solvents or devices that can mobilize or attracts the compounds.

PAH levels in solid environmental matrices can be assessed by a number of methods involving various extraction procedures. Pressurised fluid extraction (PFE) is one such technique that has been compared favourably to other extraction methods e.g. microwave, ultrasonic and Soxhlet extraction (Bjorklund *et al.*, 1998; Dean, 1998). It is common practice to affect a clean-up stage, using column chromatography containing different sorbents including alumina and florisil, post-extraction to remove extraneous material (Graham *et al.*, 2006; Li *et al.*, 2007; Wang *et al.*, 2007). However, this off-line approach is both time consuming and involves large amounts of solvents and sample manipulation. An alternate strategy has been applied recently and uses in-cell, *in-situ*, or selective PFE as a viable option for extract clean-up. The approach is a clean-up within the PFE cell facilitated by adding a specific sorbent and dispersant to the sample. The packing of the sorbent and sample within the extraction cell are of fundamental importance in the use of *in-situ* PFE (Bjorklund *et al.*, 2006; Liguori *et al.*, 2006; Canosa *et al.*, 2007; Hussen *et al.*, 2007; Rodil *et al.*, 2008; Westrom *et al.*, 2008). The comparison of the on-line and off-line procedure has shown that the former method is faster, uses less solvent and gives better recoveries (Gomez-Ariza *et al.*, 2002; Fidalgo-Used *et al.*, 2007). Therefore, the first step in the estimation of the environment and human health risk from PAHs, via the potential ingestion of

environmental matrices, was to develop a convenient and effective analytical method using PFE to identify clearly those 16 compounds. Indeed, thanks to this method, PAHs levels in any solid matrices could be quantified. Moreover, obtaining the total content of PAH in soil will be the first indispensable step towards the estimation of bioaccessibilities.

The aim of this chapter was (1) to estimate the performance of a method using a PFE system with an off-line clean-up using column chromatography, to (2) estimate the performance of the analytical method using an *in-situ* PFE method, to (3) evaluate the performance of the method using a slurry spiked soil approach, and finally to (4) observe the influence of copper on sulphur content removal in soils.

4.2 Experiment

4.2.1 Chemicals

A PAH standard solution was obtained from Thames Restek U.K Ltd., Buckinghamshire, UK (2000 µg/ml in dichloromethane). Alumina and 4,4'-difluorobiphenyl were obtained from Sigma Aldrich Ltd., Dorset, UK while florisil was purchased from Fluka (Sigma Aldrich Ltd., Dorset, UK). The copper powder was obtained from Thames Restek, UK. All the solvents (dichloromethane, acetone, hexane) were analytical reagent grade and obtained from Fisher Scientific Ltd. (Loughborough, UK). High purity diatomaceaous earth (hydromatrix) was obtained from Varian Inc. (Harbor City, CA, USA). A certified reference material (LGCQC3008 Sandy soil) was obtained from LGC Standards, Teddington, UK. The binder used in EDXRF analysis was Licowax C Micropowder PM (FLUXANA GmbH & Co, Sommerdeich, Germany). Filter papers (ASE200) made from glass fibre cellulose were obtained from Dionex Corporation (Sunnyvale, USA).

4.2.2 Instrumentation and laboratory equipment

Extractions were performed with pressurized fluid extraction (PFE) on an ASE200 instrument (Dionex UK Ltd., Camberley, Surrey) using an extraction cell of volume 11 ml. The operating conditions were organic solvent: dichloromethane: acetone (50:50, v/v); pressure: 2,000 psi; temperature: 100 ºC; and, extraction time: 10 minutes. The GC-MS instrument included a Trace GC Ultra coupled with a Polaris Q Ion trap MS (Thermo Scientific, UK) and a Triplus auto sampler injector. The system was controlled from a PC with Xcalibur™ 1.4 SR1 software. Separation was performed using a capillary column Rtx®-5MS (5 % diphenyl- 95 % dimethylpolysiloxane, 30 m x 0.25 mm ID x 0.25 µm) supplied from Thames Restek (UK). The temperature programme was as follows: start at 70 º C for 2 min and then 7 º C/ min until 180 º C, then 3º C/ min until 280 ºC, then hold for 3 min. The transfer line temperature was fixed at 300 ºC. The GC-MS operating conditions are shown in Table 4.1. The quantification of PAHs in soil samples was carried out by GC-MS using an internal standard calibration procedure. The concentration of the internal standard (4, 4'-difluorobiphenyl) was fixed at 2 µg/ml in the calibration solutions and in the spiked solutions. The standard concentration range was established from 0.5 µg/ml to 10 µg/ml, involving five calibration points. The GC-MS was operated in selected ion monitoring (SIM) mode using the ions shown in Table 4.1 for each individual PAH. All soil data were reported as PAH concentration (mg/kg, dry weight). A sonicator (Bransonic Ultrasonic Cleaner 2200) was used to warm and sonicate PAHs standards solutions before use. The X-Ray fluorescence spectroscopy apparatus was an EDXRF SPECTRO X-LAB 2000 used with the computer software X-Lab Pro 2.2.

Table 4.1: GC-MS operating conditions and acquisition parameters

Operating Conditions	Acquisition Parameter
Injector mode (GC)	Split
Carrier gas flow (GC)	1.5 ml / min
Split flow (GC)	15 ml / min
Split ratio (GC)	10
Temperature injector (GC)	280ºC
Injection volume (GC)	1 µl
Ion source temperature (MS)	270ºC
Start time (MS)	4 min
Scan mode (MS)	Selected Ion Monitoring
Damping gas flow (MS)	0.3 ml / min

GC = gas chromatography; MS = mass spectrometer

4.2.3 Soil preparation

The soil used in the spiking procedure was collected from the former Newcastle-upon-Tyne St Anthony's Lead Works, and did not show the presence of polycyclic aromatic hydrocarbons. However, blanks The soil was stored in a Kraft bag and was air-dried in a fume cupboard during one week before grinding (using pestle and mortar) below 2 mm, and sieving below 250 µm. The soil was sealed in a plastic bag, labelled and stored in the fridge (4 °C) until further analysis.

4.2.4 PFE procedure

4.2.4.1 Conventional approach

PFE and off-line clean-up: The soil sample (1.3 g) was mixed with a similar quantity of hydromatrix (Varian), and added in to the extraction cell (11 ml) on top of a filter paper. Additional hydromatrix was added to fill the cell and a final filter paper was placed on top prior to cell closure. After PFE, the solvent (dichloromethane: acetone, 1:1, v:v) was evaporated under a gentle stream of nitrogen gas to dryness and reconstituted with 2 ml of hexane. Then, the extract was treated as per column clean-up, prior to GC-MS.

Column clean-up: A column (200 mm x 18 mm) was prepared with either 10 g of Alumina (Sigma Aldrich, 150 mesh) or Florisil (Fluka, 60-100 mesh) as adsorbent with an additional 11 g of anhydrous Na_2SO_4 placed on top. Then, the column was eluted with 50 ml of hexane and the eluate was discarded. Just prior to complete elution and to avoid Na_2SO_4 powder exposure to the air, 2 ml hexane from the PFE procedure were added on top of the column for elution. (Spiking procedure: PAH standard was added: 50 µl of a 2000 µg/ml standard, in 2 ml hexane solution). Again, just prior to complete elution and dryness of the sorbent, 2 times 15 ml of hexane were added and again the eluate was discarded. Finally, the column was eluted with approximately 30 ml of dichloromethane into a flask and then the solvent was retained. Then 60 µl of the internal standard (2 µg/ml) was added to give a final volume of 30 ml.

Soil slurry spiking: A known quantity of soil (1.3 g) was placed inside a beaker. Then, 10 ml of dichloromethane containing 50 µl of the PAH standard solution (2000 µg/ml) was added to the soil. The sample was then left exposed, in a fume cupboard, for 5 days prior to PFE. After the PFE (without clean-up) the solution was reconstituted with 25 ml of dichloromethane and 50 µl of internal standard at 1000 µg/ml.

4.2.4.2 In-situ approach

2 g of Florisil or Alumina were added into the PFE extraction cell, on top of a filter paper. Then, the soil and hydromatrix were added according to the procedure described above (PFE and off-line clean-up), with a filter paper placed before closure. After *in-situ* PFE, the solvent (dichloromethane: acetone 1:1 v:v) was evaporated under a gentle stream of nitrogen gas to dryness and reconstituted with 2 ml of dichloromethane containing the internal standard (20 µl of a 1000 µg/ml solution), prior to GC-MS. In order to observe the influence of the adsorbent amount, the soils were spiked (50 µl of a 2000 µg/ml standard solution) directly in the extraction cell with 0.5 g, 1 g and 2 g of sorbent (Alumina and Florisil). In the case of no evaporation

after extraction, the final solution was reconstituted with 25 ml of dichloromethane with the internal standard (50 µl of a 1000 µg/ml solution).

4.2.4.3. Copper clean-up

To assess the effect of copper on the sulphur removal in soils and CRM, four major steps were realized. The first step was to analyse approximately 3.8 g of soil or CRM with 0.7 g of binder (Licowax C) by Energy Dispersive X-Ray Fluorescence Spectroscopy to determine the sulphur content. The second step was to mix soil with hydromatrix, as done with the PFE procedure, and check again the sulphur content by EDXRF. The third step consisted in realizing the *in-situ* PFE approach as described previously (paragraph 4.2.4.2) to assess the sulphur content of the matrix after extraction. Finally, the *in-situ* procedure was realized again, and granulated activated copper powder (2 g and 4 g) was added in the cell, above alumina, and the extract was analysed by EDXRF. Three replicates were analysed for each step and for the soil and CRM.

4.2.4.4 Certified Reference Material analysis

As part of the in-house quality control procedure a CRM was selected with PAHs of appropriate certified concentrations. In accordance with the certification of the CRM the recommended soil weight of 10 g was extracted using *in-situ* PFE with 2 g alumina.

4.2.4.5 Preliminary information on method development and validation

In order to analyse, identify and isolate PAHs from soils and more generally when analysing any compounds via an analytical procedure, there are essential steps required to control the quality of the results and improve the performance of the analytical method. For instance, when using a GC-MS for the analysis of pollutants in environmental matrices, several parameters and tools can be used to control the quality:(i) Selection of ions with mass spectrometer parameters: either full scan or SIM (Selected ion monitoring), (ii) Choice of concentration range for calibration curves, and

choice of the type of calibration (internal, external, standard addition), (iii) Time of analysis, temperature program, transfer line temperature, ion source temperature, choice of the amount injected in GC,(iv) Choice of the injection port, (v)Preparation and injection of blank samples, duplicates, spiked samples and standard checks during analysis, certified reference material during the extraction, (vi) Auto sampler parameters.

With these parameters, a method development can be realized on a GC-MS with a PAH standard solution. Developments and observations are still made when doing extractions, by taking care of the sample solution injected (clean-up), the appearance of chromatograms after injections (peak-tailing and column-bleeding), and the consistency of standard quantitations when analysing samples. Then, the entire analytical procedure with extraction can be developed according to observation of recoveries with spiking procedure. Indeed, in the comparison of off-line and on-line clean-up, spikes are realized to estimate the % recovery and precision of the results. However, other parameters are observed, such as the efficiency of the clean-up according to the colour of the extract, the influence of the adsorbent, and the effect of specific compounds to remove impurities from soils. After being developed, a method is used to quantify the contaminants in unknown samples, for example with PAHs in solid environmental matrices.

4.3 Results and Discussion

4.3.1 Example of chromatogram with 16 PAHs

After optimizing the parameters of the GC-MS, a suitable temperature program was found for the analysis of 16 PAHs in less than 60 minutes. Peaks were sharp, isomers were well separated, and peaks were in good intensities for a 5 µg/ml concentration (Figure 4.1).

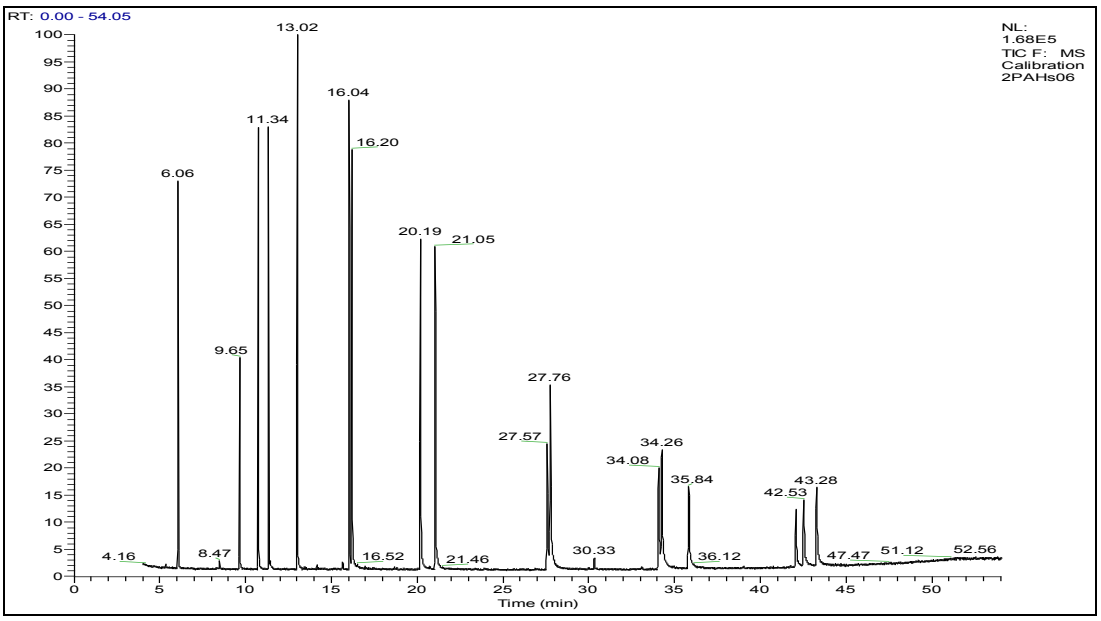


Figure 4.1: Chromatogram of 16 PAHs at 5 µg/ml concentration with conditions stated in experimental part

4.3.2 Analytical figures of merit

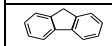
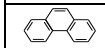
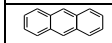
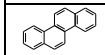
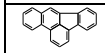
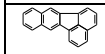
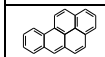
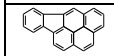
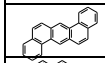
Initial work established the basic analytical figures of merit for quantifying PAHs using GC-MS with typical calibration curve correlation coefficients >0.995 (Table 4.2).

4.3.3 PFE procedure

4.3.3.1 Conventional approach

PFE followed by off-line clean-up with both adsorbents gave average recoveries for mid-molecular weight PAHs (fluorene to pyrene) of approximately 80 % whereas for the heavier molecular weight PAHs i.e. benzo(a)anthracene to benzo(ghi)perylene the average recoveries were typically 50%. For the lightest, i.e. small molecular weight PAHs, recoveries of < 5 % for naphthalene, < 30 % for acenaphthylene and < 40 % for acenaphthene were obtained (Figure 4.2). Typical SDs for the recovery of PAHs, using alumina and florisil, ranged from 11.1 to 61.4 % and 3.3 to 68.9 %, respectively (Lorenzi *et al.*, 2008).

Table 4.2: GC-MS calibration of PAHs based on a five point graph (0.5 - 10 µg/ml)

PAH Structure	Empirical Formulae	PAHs	Retention time ($t_{R;}$ min)	MS Ion for Quantitation	Calibration Regression y = mx + c	Correlation Coefficient R^2
	C10H8	Naphthalene (NAP)	6.06	128	4.1399 X + 0.7205	0.9986
	C12H8	Acenaphthylene (ACY)	10.95	152	4.1139 X + 0.0279	0.9999
	C12H10	Acenaphthene (ACE)	11.34	154	2.3134 X + 0.1547	0.9993
	C13H10	Fluorene (FLU)	13.02	166	2.9124 X + 0.037	0.9998
	C14H10	Phenanthrene (PHE)	16.04	178	4.5264 X + 0.0952	0.9995
	C14H10	Anthracene (ANT)	16.20	178	4.2730 X - 0.2848	0.9999
	C16H10	Fluoranthene (FLUH)	20.19	202	4.5104 X - 0.8234	0.9996
	C16H10	Pyrene (PYR)	21.05	202	4.8043 X - 0.7057	0.9998
	C18H12	Benzo(a)anthracene (BaA)	27.57	228	2.9000 X - 0.9132	0.9974
	C18H12	Chrysene (CHY)	27.76	228	4.4652 X - 1.6144	0.9969
	C20H12	Benzo(b)fluoranthene (BbF)	34.06	252	2.7100 X - 0.8907	0.9972
	C20H12	Benzo(k)fluoranthene (BkF)	34.26	252	3.6894 X - 1.4761	0.9954
	C20H12	Benzo(a)pyrene (BaP)	35.84	252	2.6269 X -0.9960	0.9955
	C22H12	Indeno(1,2,3-cd)pyrene (IDP)	42.09	276	4.0229 X - 1.7347	0.9977
	C22H14	Dibenzo(a,h)anthracene (DBA)	42.53	278	4.7652 X – 2.3214	0.9970
	C22H12	Benzo(g,h,i)perylene (BgP)	43.26	276	5.6479 X - 2.7142	0.9973

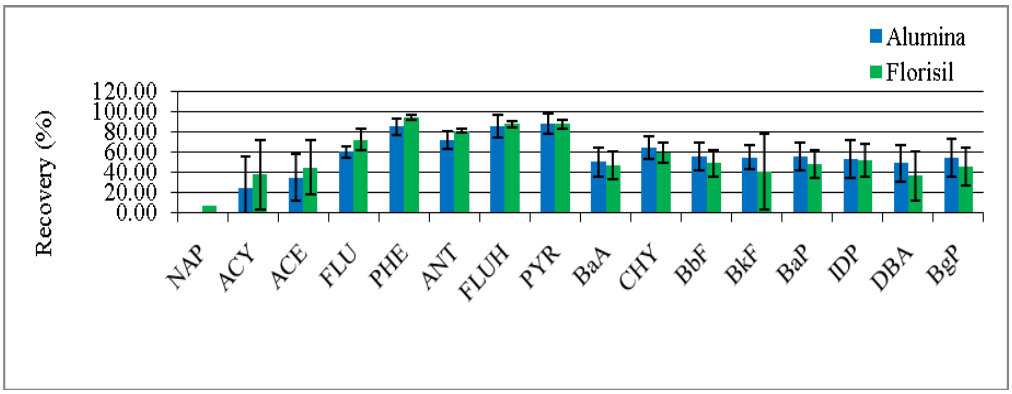


Figure 4.2: Recoveries of PAHs after PFE with off-line clean-up (mean +/- sd, n = 3)

4.3.3.2. Adsorbent amount influence

Various amounts of adsorbent were inserted in the cell of the PFE in order to find the best quantity to add to realize the clean-up of the extract whilst at the same time obtain efficient recoveries from PAHs after extraction. The *in-situ* PFE-GC-MS procedure was done as described in the experimental procedure, with different amounts of adsorbents (0.5 g, 1 g and 2 g). According to Figures 4.3 and 4.4 the most convenient amount of adsorbent to get recoveries between 75 % and 120 % was 2 g. Moreover, the results were precise because relative standard deviations were below 20 % for 2 g of adsorbent. There was not a significant variation between alumina and florisil. Alumina was chosen for the rest of the studies because the overall results were more consistent and efficient.

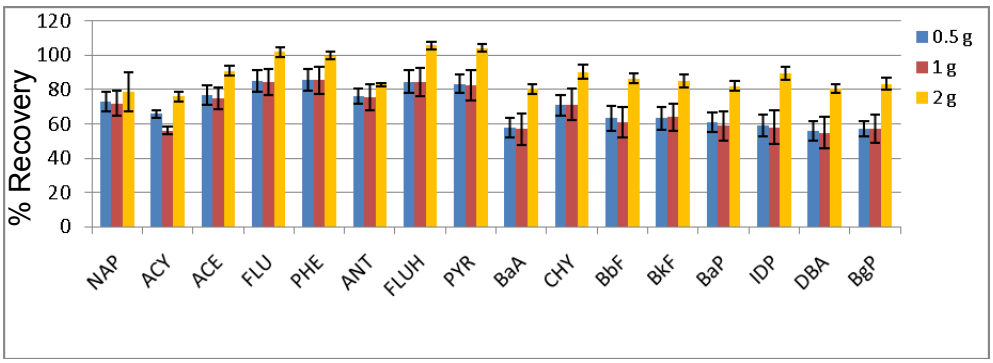


Figure 4.3: Recoveries of PAHs after PFE with in-situ clean-up (mean +/- sd, n = 3) with three different amount of Florisil (0.5, 1 and 2 g)

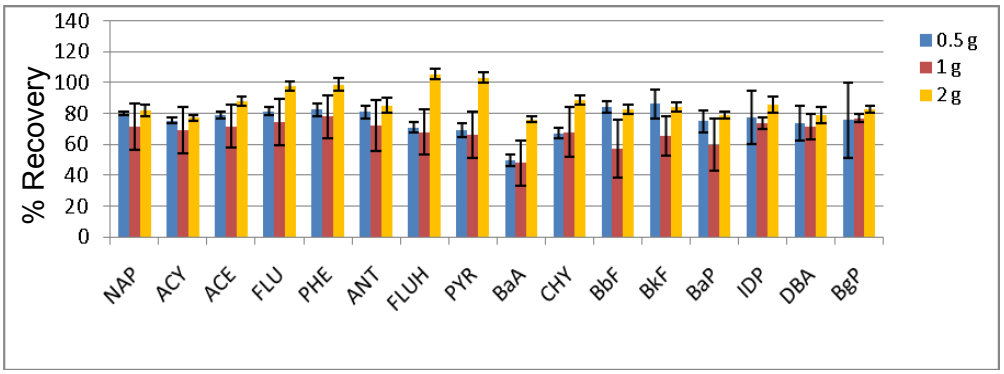


Figure 4.4: Recoveries of PAHs after PFE with *in-situ* clean-up (mean +/- sd, n = 3) with three different amount of Alumina (0.5, 1 and 2 g)

4.3.3.3. Spiking procedure of the in-situ approach

Soil samples were spiked directly into the PFE cell to assess the impact on PAH recovery using *in-situ* clean-up with either alumina or florisil. It can be seen in Figure 4.5 that good recoveries (~90 %) were obtained for all PAHs when no further sample concentration took place (no solvent evaporation post-extraction). Typical RSDs for the recovery of PAHs, using alumina and florisil, ranged from 4.0 to 10.5 % and 1.1 to 22.4 %, respectively. No specific influence is noted in terms of the use of florisil and alumina on recovery of PAHs. This is not the case in Figure 4.6 in which post-extraction evaporation under a stream of N_2 results in significant losses of naphthalene (>80%), and to a smaller extent for acenaphthylene and acenaphthene.

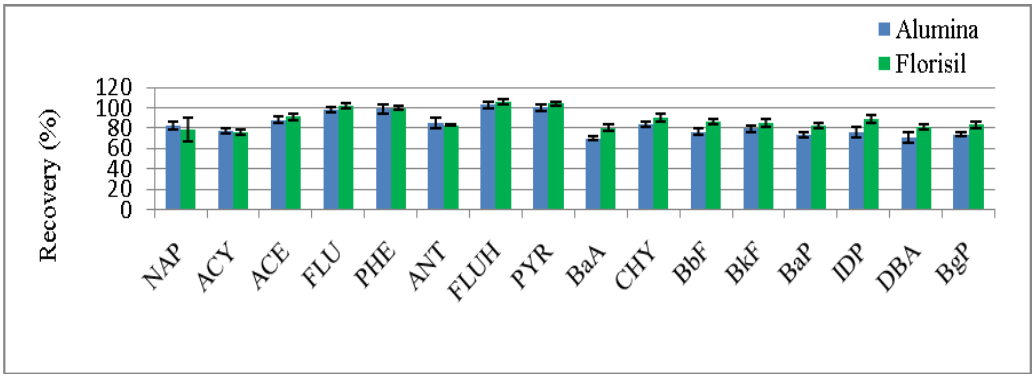


Figure 4.5: Recoveries of PAHs after PFE with *in-situ* clean-up without evaporation (mean +/- sd, n = 3)

Appropriate recoveries are noted for alumina for the other PAHs whereas elevated recoveries are noted for the mid-range PAHs when using florisil as the *in-situ* adsorbent. Typical RSDs for the recovery of PAHs, using alumina and florisil, ranged from 2.7 to 25.7 % and 3.8 to 22.2 %, respectively.

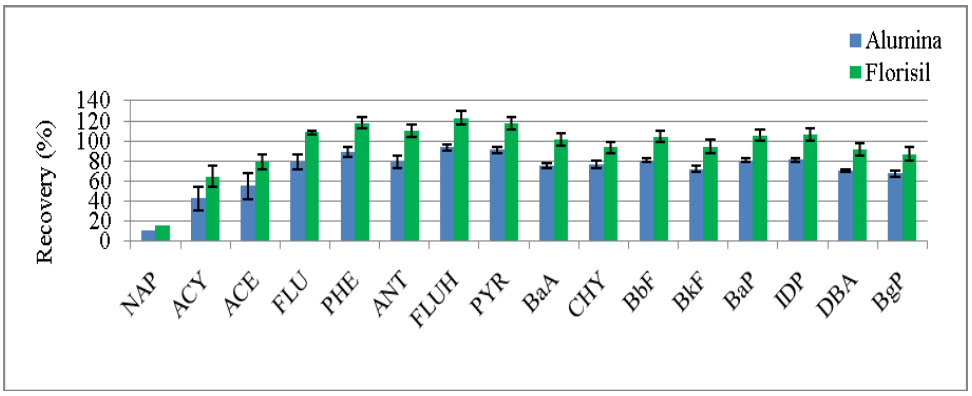


Figure 4.6: Recoveries of PAHs after PFE with *in-situ* clean-up with evaporation (mean +/- sd, n = 3)

The process was repeated using PAH slurry spiked soil. It is shown in Figure 4.7 that the overall recovery of PAHs was significantly reduced (~50 %) using this soil spiking approach. While higher recoveries are noted for alumina the major losses are most likely due to evaporation of the PAHs during the 5 days equilibration period. Typical RSDs for the recovery of PAHs, using alumina and florisil, ranged from 3.7 to 10.3 % and 8.7 to 24.8 %, respectively (Lorenzi *et al.*, 2008).

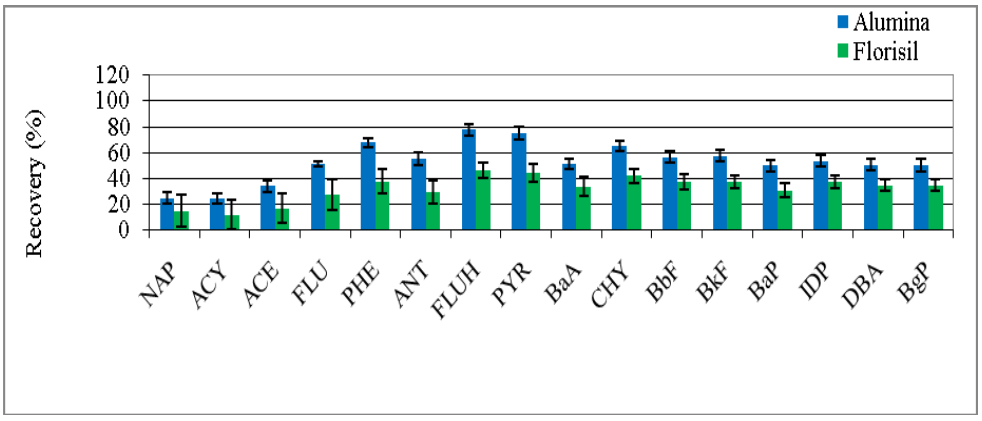


Figure 4.7: Recoveries of PAHs from a slurry spiked soil after PFE with *in-situ* clean-up (mean +/- sd, n = 3)

4.3.3.4. Comparison of conventional and in-situ approach

After evaluating each approach for isolating PAHs from soils, the optimum procedure was described as follows: PFE with *in-situ* clean-up using 2 g alumina and without evaporation after extraction. According to the overall results, florisil appeared to be less efficient than alumina. The quantity of adsorbent seemed enough with 2 g to obtain good recoveries (more than 80 %). The evaporation step should be preferably avoided because some PAHs are lost. The more volatile PAHs, especially naphthalene, acenaphthylene and acenaphthene, were evaporated because of their low molecular weight. Regarding the efficiency of the clean-up, it was worthwhile observing the colours of the extracts. With the offline clean-up (10 g of sorbents) the extract colour was very transparent (Figure 4.8) showing that clean-up was effective. Considering the *in-situ* clean-up with 2 g, it produced a slightly brown solution whereas the same experiment with evaporation showed a dark-brown colour (Figure 4.8). There were no significant differences in colour between 0.5, 1 and 2 g using the *in-situ* clean-up (Figure 4.9). However, 2 g gave better recoveries and a slightly more light-brown colour (Figure 4.8). Therefore, 2 g integrated in an *in-situ* PFE-GC-MS procedure seemed a reliable method to replace an off-line clean-up. The soil slurry spiking approach showed that PAHs were very sensitive to loss due most likely to evaporation, as concentration are significantly lower after that the spiked soil was exposed to the air during several days.

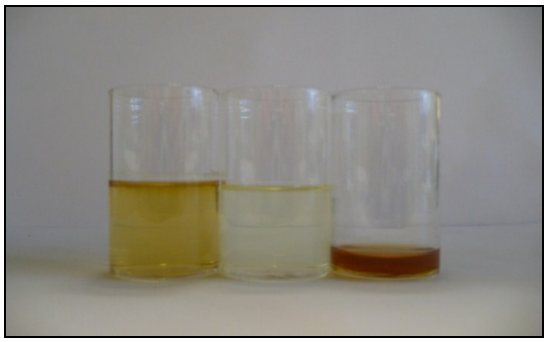


Figure 4.8: Colour of the extract after *in-situ* PFE, off-line PFE and *in-situ* with evaporation using 2 g of alumina (from left to right)

Figure 4.9: Colour of the extract after *in-situ* PFE with 0.5 g alumina and florisil, and with 1 g alumina and florisil (from left to right)

4.3.4. Copper influence

The sulphur percentage in soil and CRM was estimated as a function of the copper powder added in the cell of PFE system and analysing the extract by XRF (Figure 4.10). The copper powder had no effect, or very negligible, on the sulphur content removal of the soil sample and CRM, in this study. The bar charts showed that either with/ without copper powder the percentage of sulphur content stayed the same in the soil. The mean sulphur content in the soil was 28 % and remained constant at 29 % and 28 % by adding 2 g or 4 g of copper, respectively. The mean sulphur content in the CRM was 14 % and became 15 % after adding 2 g of copper before pressurized fluid extraction. The only difference noticed was that the mixing with hydromatrix (Blank) reduced the sulphur content in soil. The Mean sulphur content in the pure soil and pure CRM was 55 % and 50 %, and after mixing with hydromatrix the mean sulphur content was 33 % and 26 %. But it could be only an effect of reducing the amount of soils analysed by the XRF, because mixed (1:1, w:w) with hydromatrix.

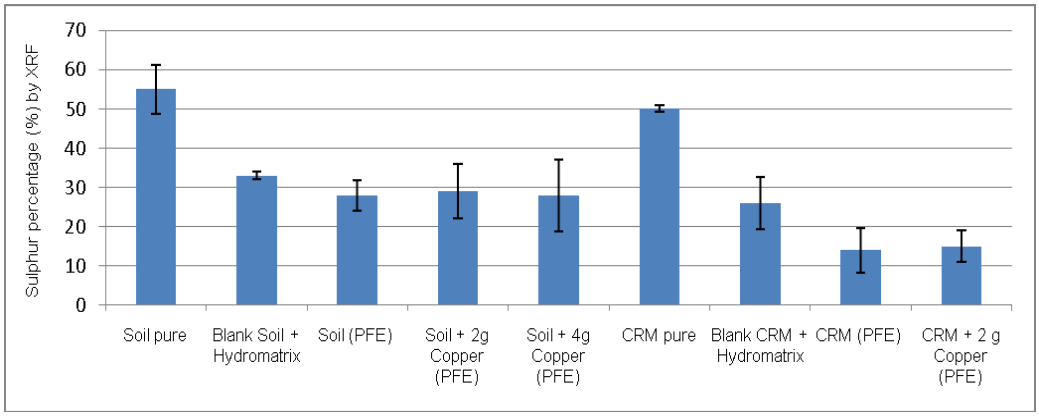


Figure 4.10: Sulphur percentage in soil and CRM as a function of copper amount after PFE and XRF analysis (mean +/- sd, n = 3) (CRM: Certified Reference Material)

4.3.5 Analysis of a Certified Reference Material

All CRM results were reported within the certified values (+/- standard deviation), except dibenzo(a, h)anthracene where the measured value was above the indicative value of < 2 mg/kg (Table 4.3). As the concentration of dibenzo (a,h) anthracene was only an indicative value and not a certified value no further investigation was considered necessary.

Table 4.3: Determination of PAHs in a certified reference material (CRM LGC QC 3008) using *in-situ*-PFE-GC-MS

PAHs	CRM LGC QC 3008 (sandy soil 2)	
	Measured (+/- SD) n = 3 (mg/kg)	**Certificate Value (+/- SD) n = 3 (mg/kg)**
Naphthalene	3.4 ± 0.1	3.1 ± 0.9
Acenaphthylene	3.9 ± 0.5	3.4 ± 1.6
Acenaphthene	1.5 ± 0.3	<2
Fluorene	6.7 ± 0.4	7.7 ± 1.7
Phenanthrene	28.7 ± 3.8	34 ± 7.1
Anthracene	8.0 ± 0.8	5.9 ± 2.1
Fluoranthene	29.2 ± 6.0	32 ± 6.4
Pyrene	20.6 ± 3.5	24 ± 6.5
Benzo(a)anthracene	10.2 ± 1.8	11 ± 2.5
Chrysene	9.1 ± 1.1	9.9 ± 2.1
Benzo(b)fluoranthene	10.4 ± 1.8	9 ± 3.3
Benzo(k)fluoranthene	6.1 ± 1.3	5.8 ± 2.2
Benzo(a)pyrene	8.3 ± 1.5	8.2 ± 1.8
Indeno(1,2,3-cd)pyrene	6.6 ± 1.4	5.2 ± 1.8
Dibenzo(a,h)anthracene	3.7 ± 0.2	<2
Benzo(g,h,i)perylene	6.1 ± 1.1	5.2 ± 1.8

4.4 Conclusion

An analytical method was developed to separate and identify the 16 priority pollutant PAHs from solid environmental matrices. An *in-situ* PFE-GC-MS method with 2 g of alumina for the clean-up was discovered to be suitable to analyse the 16 PAHs that are potentially contained in numerous solid environmental matrices. This new way of doing the purification and extraction in only one step was shown to be very effective compared to an off-line mode using column chromatography. A comparison between the *in-situ* approach, with and without evaporation at the end of the process,

demonstrated the strong tendency of low molecular weight PAHs to evaporate, due to their high volatility, which should be considered in further work with those compounds. The off-line approach was not showing satisfactory recoveries with the spiking procedure. A slurry spiking procedure correlated this observation, by showing significant losses of PAHs after leaving a soil in a slurry for a couple of days. Copper powder showed no effect in removing sulphur in soil. Therefore copper powder will not be included in future extractions. This method shows that validating an analytical method requires the analysis of several parameters such as precision, accuracy, repeatability, sensitivity and selectivity. The comparison of the PAH concentrations obtained with a certified reference material and its certificate values also demonstrate the quality of our results, considering the instrument and operator of the present laboratory. Therefore, this method will be ideal for the rest of this project and for other studies requiring the identification of PAHs in solid environmental matrices. Particularly in this study, this method will help finding the total PAH content in contaminated soils or road dusts, in order to establish the risk for the environment and for humans regarding the levels of contaminants potentially ingested via these matrices. This extraction will also be used in relation with physiologically-based extraction tests, to compare values, in the solid environmental matrices and in the gastrointestinal fluids, in order to evaluate bioaccessibilities.

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Chapter 5: An investigation into the occurrence and distribution of PAH, in two soil size fractions, on a former industrial site, NE England, UK using *in-situ* PFE-GC-MS

5.1 Introduction

PAHs can be introduced into the environment via incomplete organic matter combustion at high temperature (pyrogenic origin), oil spill and natural oil leakage (petrogenic origin) and via natural precursor transformations during early diagenesis processes (Mazeas *et al.*, 1999). The pyrogenic sources, mainly higher molecular weight PAHs, result primarily from human and industrial activities. The petrogenic sources, generally lower molecular weight PAHs, include organic-rich shales and natural oil seeps (Neff *et al.*, 2003). Therefore, analyses of PAHs in soils from both anthropogenic and natural sources are relevant in the study of their occurrence and distribution in the environment.

The former UK soil total PAH trigger concentration for land used as domestic gardens, allotments and play areas was 50 mg/kg (ICRCL, 1987). However, these guidelines were withdrawn in 2002. The Environment Agency (EA) in England and Wales is currently in the process of producing new Soil Guideline Values (SGVs) for PAHs (EA, 2009). Therefore, at the present time there are no published SGVs for the PAHs. In the absence of SGVs, the generally accepted limit for landscaping and domestic garden soil for benzo(a)pyrene is 1 mg/kg and for total PAHs is 40 mg/kg (Tim O'Hare Associates, 2002). The 40 mg/kg value is based on the Dutch intervention value which is itself based on the sum of ten individual PAHs i.e. naphthalene, anthracene, phenanthrene, fluoranthene, benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(ghi)perylene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene (VROM, 2000). As part of the contaminated land regulatory regime in England and Wales, Generic Assessment Criteria (GAC) have been published for benzo(a)pyrene, fluorene, naphthalene, and dibenzo(a,h)anthracene (Nathanial *et al.*, 2007). The GAC identified

values between 1.08 and 1.32 mg/kg dry weight for benzo(a)pyrene for residential land use (with/without plant uptake) which concur with the generally accepted limit of 1 mg/kg (Nathanial *et al.*, 2007). Above these values it is considered to be a potential risk in the environment, for human health. It should also be noted that all these guidelines are based on a < 2 mm soil size fraction (EA, 2009).

In chapter 4, we compared the use of different quantities of alumina and florisil with / without the presence of copper powder (for removal of sulphur) for the analysis of PAHs from soil (Lorenzi *et al.*, 2008). Results showed that 2 g of alumina was appropriate for effective extract clean-up with no requirement for the addition of copper powder, as adding copper in the PFE cell was not removing efficiently the sulphur content. In addition, the work compared the benefits of off-line and on-line PFE for the recovery of PAHs from soils. *In-situ* PFE provided a viable alternative to extraction of PAHs from soils that was fast, efficient and minimised the use of organic solvent (Lorenzi *et al.*, 2008). Other workers have compared both on-line and off-line procedures for post-extraction clean-up with similar results (Gomez-Ariza *et al.*, 2002; Fidalgo-Used *et al.*, 2007).

Oral ingestion of soils, either intentionally or unintentionally, is an important exposure route for contaminants from the environment to humans. Ingestion can take place by consumption of unwashed fruits and vegetables; from hand-to-mouth contact when children are playing on the floor, by drinking water filtered through soils, via pica-soil behaviour and through poor personal hygiene (Intawongse *et al.*, 2006). It has been reported (Bornschein *et al.*, 1987) that the < 250 μm soil size fraction is most representative of the grain size that adheres to an individual's hands; hence it represents a primary source of soil ingestion. Therefore, when undertaking environmental risk assessment, it is important that the distribution of PAHs in the < 250 μm soil fraction is considered.

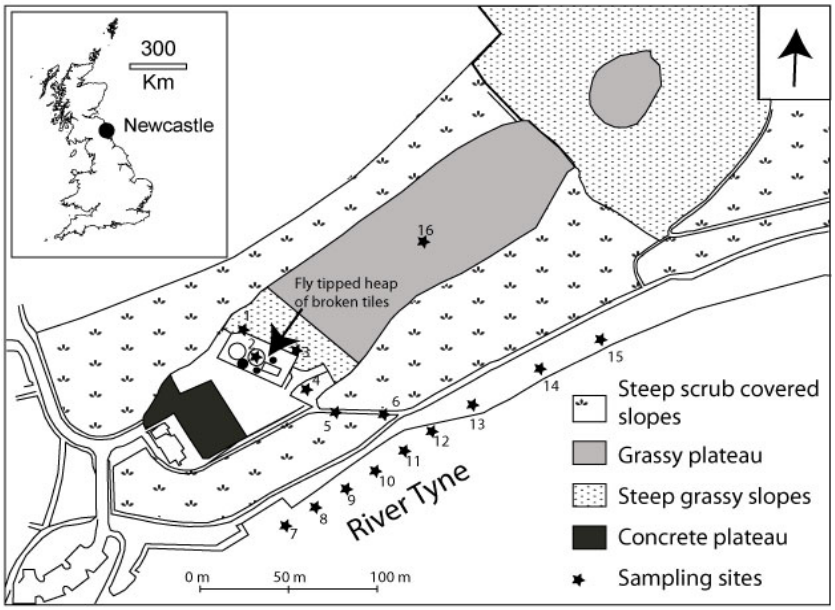
To test the determination of PAHs in different soil size fractions using the PFE method, the former St Anthony's Tar Works, in Newcastle-upon-Tyne (NE England, UK) was selected as the study area. This study area was representative of historically contaminated land that was under-developed since closure of the industry in 1981, having previously been in operation since the 1920s. Whilst in business, the Works generated a variety of products distilled from imported coal tar, including tar, pitch, naphtha, anthracene, creosole, benzol, and cyanide which were stored in tanks on the site (P Hartley, Newcastle City Council, Personal Communication, 2009). The purpose of this research was to (1) apply *in-situ* PFE to assess the level of PAH contamination in the study area, (2) evaluate whether PAHs were distributed within different soil particle sizes uniformly, and (3) to assess the implications of the PAH – soil particle size relationship in terms of environmental human health risk.

5.2 Experimental

5.2.1 Sampling procedure

The soil sampling was undertaken at the former St Anthony's Tar Works in October 2008 (Figure 5.1). Soils were taken from 16 locations within the study area: on the upper plateau (sampling sites 1 - 4), the slope down to the River Tyne (sampling sites 5 - 6), the foreshore close to the river (sampling sites 7 -15), and finally a potential historic dumping site close to the former Tar Works (sampling site 16) (Figure 5.1). More stringent safety precautions were taken when soil sampling on the foreshore of the river due to the strong hydrocarbon odour emanating from the area. Precautions included the wearing of disposable masks and gloves and Wellington boots that could be de-contaminated. The soils were manually collected (top 0 - 10 cm soil layer) and transported back to the laboratory in Kraft® paper bags. In the laboratory, soil samples were air-dried at room temperature (< 20 °C) in a fume cupboard (Barnabas *et al.*, 1995a; Barnabas *et al.*, 1995b), prior to grinding (using a pestle and mortar) and sieving (using a plastic sieve) to soil size fractions of < 2 mm and < 250 µm. Both soil

particle size fractions (fraction A: < 250 µm and fraction B: > 250 µm to < 2 mm) were sealed in plastic bags, labelled and stored in the fridge at 4 ºC until required for further analysis. As the GC-MS was operated in selected ion monitoring mode, no contamination from plastic ware was identifiable in the analysis.



The study area is located at UK National Grid co-ordinates NZ 291 631.

Figure 5.1: Soil sampling plan and location of the St Anthony's Tar Works study area, Newcastle upon Tyne.

5.2.2 Analysis

All the soil samples were analysed for the 16 priority PAHs outlined in Table 4.2 (chapter 4). Each PAH was extracted by *in-situ* PFE followed by Gas Chromatography Mass Spectrometry (GC-MS), as described in chapter 4. Chemicals, figures of merits and certified reference material results were also listed in chapter 4, so they are not represented in this chapter.

5.2.3 *In-situ* PFE protocol

The *in-situ* PFE procedure was realized with 2 g of soil sample instead of 1.3 g in the chapter 4, with the method development. The experimental procedure remains the same, apart from this change in the mass of soil. After PFE, the solvent

(dichloromethane : acetone, 1:1, v/v) was evaporated under a gentle stream of nitrogen gas to dryness and reconstituted with 2 ml of dichloromethane containing the internal standard (20 µl of 1000 µg/ml solution), prior to the injection of 1 µl in the GC-MS.

5.2.4 Determination of soil pH

The pH was determined in a soil: distilled water suspension 1: 2.5 w/v (Strowbel *et al.*, 2005) as follows; 10 g of soil sample was accurately weighed into a small beaker and 25 mL of distilled water was added to the soil. The sample was shaken and stirred for 5 minutes. Then, the sample was left to stand for 10 minutes and the pH recorded. The pH was measured using a pH meter after being calibrated with buffer solutions of pH 4 and 7.

5.2.5 Determination of soil organic matter content

Soil organic matter content was determined using the method of loss of ignition (LOI) (Baize, 1993). 5 g of soil sample was accurately weighed into a pre-weighed crucible. The weight of soil (W) and the weight of soil and crucible (W1) were recorded. The sample was placed in a pre-heated muffle furnace (800 °C) for half an hour and then removed from furnace with gloves to be cooled in a desiccator. The sample was re-weighed and the weight was recorded (W2). The % LOI was calculated using the equation below:

$$\% \text{ LOI} = (W1 - W2) \times 100 \qquad [5.1]$$

5.3 Results and discussion

5.3.1 Preliminary information

It should be noted that previous work in our laboratory has shown that evaporation to dryness under a stream of nitrogen post- *in-situ* PFE results in loss of the most volatile PAH (i.e. naphthalene) by as much as 80 % (Lorenzi *et al.*, 2008). In addition, losses due to solvent evaporation were noted for acenaphthylene (50 %), acenaphthene (35

%) and fluorene (15 %). For higher molecular weight PAHs, no significant differences were noted with/ without evaporation post *in-situ* PFE. Therefore, these aspects of the analytical method have to be considered when interpreting the results.

5.3.2 Soil total PAH concentrations

An initial assessment of the average total PAH concentrations in the 16 soil samples, revealed ranges from 9.0 to 1404 mg/kg in soil fraction A and from 6.6 to 872 mg/kg in soil fraction B (Table 5.1). The results also showed that the majority of the samples, irrespective of soil particle size, had a total PAH concentration above the generally agreed threshold for total PAHs of 40 mg/kg (Tim O'Hare Associates, 2002).

The results from the present study were compared to total PAH concentrations in soils from selected industrial sites around the world (Table 5.2). The St Anthony's Tar Works soils showed a wider range in total PAH concentrations than most of the values reported from elsewhere in the literature. Although some very high total PAH concentrations have been reported in the vicinity of an oil refinery in Belgium (300 mg/kg), an aluminium smelter in Slovakia (200 mg/kg) and a chemical plant in Australia (79 mg/kg), the majority of published soil total PAH data from industrial sites fall within the range of 0.1 to 18 mg/kg (Table 5.2). The higher soil total PAH concentrations (6.6 to 1404 mg/kg) recorded in the present study indicate that the St Anthony's Tar Works site is significantly contaminated and warrants further investigation/ remediation as it may represent an environmental and human health risk. A statistical comparison (t-test) of the mean total PAH concentrations, in soil fractions A and B, indicated that there were significant differences (95% confidence interval) in 14 out of the 16 soils. The exceptions to this were soil samples 5 and 6 for which no statistical difference in total PAH concentration was evident between the two soil fractions (Table 5.1).

Table 5.1: Average total PAH concentrations and t-test comparison for the two soil size fractions from the St. Anthony's Tar Works study area.

	Sampling Site															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Total PAHs (mg/kg)(soil fraction A)*	123 ± 22	9.0 ± 0.2	1404 ± 73	366 ± 22	66.5 ± 1.4	46.4 ± 1.4	38.9 ± 0.4	40.5 ±1.9	375 ± 34	289 ± 8	54.1 ± 2.1	43.6 ± 2.5	41.6 ±1.0	40.8 ± 0.7	43.7 ± 0.8	39.7 ± 0.7
Total PAHs (mg/kg)(soil fraction B)*	234 ± 17	6.6 ± 0.1	872 ± 176	285 ± 10	69.2 ± 10.3	39.9 ± 4.7	23.6 ± 0.8	65.8 ± 0.7	173 ± 4	585 ± 39	88.7[#]	59.0 ± 4.8	30.6 ± 3.9	38.8[#]	28.4 ± 2.2	28.3 ± 2.2
t-test value ($t_{critical}$ = 2.78)	**-6.90**	**18.2**	**4.83**	**5.78**	-0.44	2.27	**29.8**	**-21.6**	**10.19**	**-12.9**	**-28.5**	**-4.92**	**4.73**	**5.04**	**11.4**	**8.57**

*** Mean of three analyses (± SD)**

[#] n = 1 only (limited sample available).

Soil fraction A = < 250 µm and soil fraction B = > 250 µm < 2 mm

The figures in bold represent the statistically significant values (above $t_{critical}$) (95% confidence interval), of comparisons between mean total PAH concentrations in soil fractions A and B.

Table 5.2: Total PAH concentrations in urban soils from selected industrial sites in a range of different countries compared to the present study.

Country	ΣPAHs (mg/kg dry weight)	Number of PAHs Analysed	Source	Soil Depth (cm)	Reference
Australia	0.3-79	18	Vicinity of a chemical plant	0-5	(Weiss *et al.*, 1994)
Austria	1.45*	18	Industrial area	ND	(Weiss *et al.*, 1994)
Belgium	300 (50 m away); 3-14 (1.3-4.2 km away)	7	Vicinity of an oil refinery	ND	(Bakker *et al.*, 2000)
Brazil	0.1	20	Vicinity of industrial activities	ND	(Wilcke *et al.*, 1999a)
China	0.82 ± 0.80	16	Industrial area	ND	(Wang *et al.*, 2003)
Estonia)	12.39 ± 9.81	16	Oil-shale thermal treatment industry, power station and traffic	ND	(Trapido, 1999)
France	0.45-5.65	14	Near industrialised area	ND	(Motelay-Massei *et al.*, 2004)
Germany	10.2	20	Vicinity of industrial activities	ND	(Wilcke *et al.*, 1997)
Ghana	0.1	20	Periurban agricultural soils	ND	(Wilcke, 2000)
Greece	0.55-4.95	16	Lignite-fired power plants	ND	(Stalikas *et al.*, 1997)
Japan	1.3 ± 0.8	8	Vicinity of industrial activities	0-3	(Spitzer *et al.*, 1993)
Korea	0.16*	16	Agricultural soil	ND	(Nam *et al.*, 2003)
Slovakia	40-200	17	Vicinity of an aluminium plant	Surface+	(Wilcke *et al.*, 1996)
Spain	1.00 ± 1.52	16	Near chemical industries	ND	(Nadal *et al.*, 2004)
Switzerland	11 ± 12	16	Vicinity of industrial activities	0-20	(Niederer *et al.*, 1995)
Thailand	0.1	20	Vicinity of industrial activities	ND	(Wilcke *et al.*, 1999b)
UK	12-18	16	Vicinity of industrial activities	0-10	(A.A. Meharg *et al.*, 1998)
USA	3.73*	16	Vicinity of industrial activities	ND	(Mielke *et al.*, 2001)
Newcastle-upon-Tyne (NE England)	6.6 – 1404	16	Former tar works site	0-10	Present Study

* median value

+ organic surface layer

5.3.3 Soil individual PAH concentrations

The relationships between PAH concentrations in soil fractions A and B were examined further for the 16 individual PAHs determined during the study. A plot of individual PAH concentrations in the 16 soil samples revealed that the majority of PAHs were higher in concentration in soil fraction A (< 250 µm) (Figure 5.2). This was particularly true of the highest concentrations (> 100 mg/kg) of some individual PAHs (e.g. fluoranthene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene (Figure 5.2). Some exceptions to this trend i.e. higher individual PAH concentrations (> 100 mg/kg) in soil fraction B, were noted for phenanthrene and fluoranthene (Figure 5.2). In addition, if all the individual PAH determined i.e. 16 PAHs x 16 soil samples x 2 soil size fractions were summed (i.e. 512 individual PAH concentrations) the percentage of results that are higher in soil fraction A is 65.8 %.

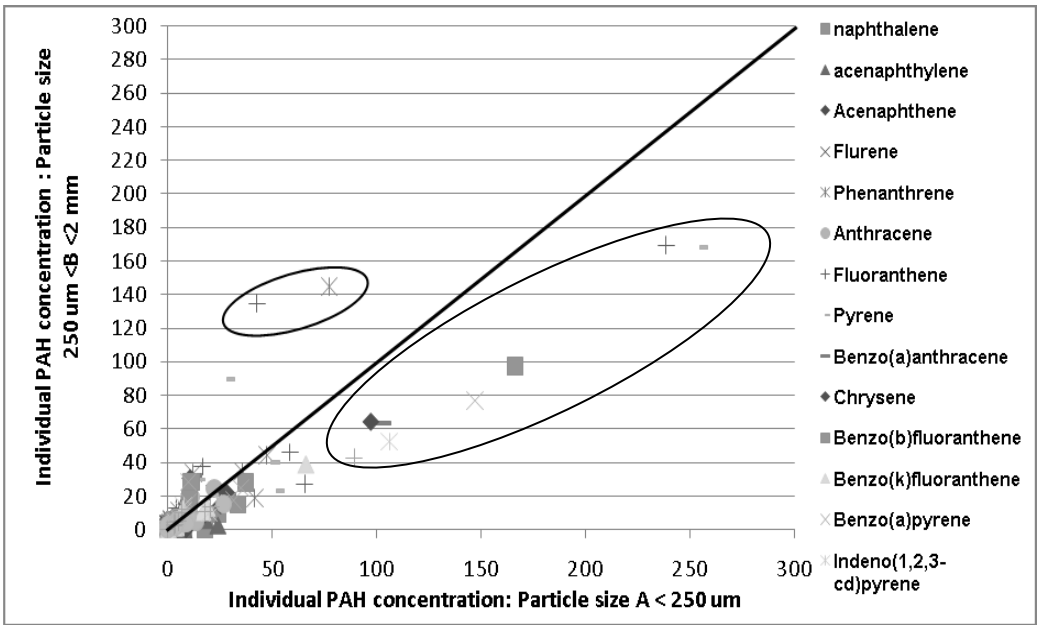


Figure 5.2: The influence of soil particle size on the concentration of individual PAHs: PAH concentration (mg/kg) in particle size B as a function of particle size

Furthermore, statistical comparison (t-test) of all the soil samples confirmed that 68.9% of the mean individual PAH concentrations were significantly different (95% confidence interval) between soil fractions A and B (Table 5.3).

Table 5.3: Statistical (t-test) comparisons between two soil size fractions for 16 individual PAH concentrations from the St. Anthony's Tar Works' study area.

PAH abbreviation	Sampling Site															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
NAP	**-2.79**	ND	**11.5**	-0.05	-0.37	**43.7**	**33.0**	**9.67**	**20.9**	**31.6**	**-40.9**	-0.81	**32.1**	**15.9**	**30.2**	**7.47**
ACY	-1.15	ND	**3.26**	1.54	2.65	**39.7**	**180**	**28.8**	**112**	**-3.73**	11.1	**6.17**	**46.7**	**62.2**	**258**	**30.7**
ACE	ND	ND	**17.1**	1.04	ND	ND	ND	ND	**5.91**	**6.98**	ND	ND	ND	ND	ND	ND
FLU	**-4.43**	ND	1.12	-1.73	ND	**-22.5**	**-251**	**-3.31**	**8.69**	0.91	ND	-1.99	ND	ND	ND	ND
PHE	**-5.63**	-2.49	0.66	0.38	-1.11	-2.19	2.00	-1.40	**9.53**	**-20.6**	**-34.6**	**-16.2**	**-18.5**	**-34.5**	**-7.00**	**-12.7**
ANT	**-3.34**	**89.9**	-0.37	-1.00	-1.62	0.67	**18.3**	-0.50	**8.53**	**-6.89**	**-110**	-2.34	**-6.59**	**-31.31**	**-3.70**	**9.61**
FLUH	**-7.51**	2.01	**4.25**	**4.59**	-0.71	-1.65	1.52	-1.09	**13.4**	**-10.3**	**-24.4**	**-13.2**	1.43	**20.4**	**4.71**	2.71
PYR	**-9.10**	0.14	**4.68**	**5.85**	-0.73	0.11	**17.8**	-0.97	**7.58**	**-12.4**	**-58.6**	**-4.96**	1.27	**16.4**	**3.95**	**4.10**
BaA	**-8.33**	ND	**4.16**	**5.84**	-0.18	**4.25**	**19.3**	-0.37	**6.77**	**-10.4**	**-7.37**	1.46	**7.62**	0.64	**30.1**	**14.4**
CHY	**-5.39**	ND	**3.87**	**5.37**	-0.65	2.24	2.67	-0.99	**13.5**	**-6.50**	**-40.7**	-1.44	**10.9**	**207**	**67.3**	**13.4**
BbF	**-6.42**	ND	**4.23**	**4.30**	-0.61	**4.32**	**10.0**	-0.44	**9.16**	**-8.58**	**-14.9**	-1.19	**6.71**	**-46.9**	**4.74**	2.31
BkF	**-5.45**	ND	**3.32**	**6.54**	-0.65	**10.8**	**12.6**	0.19	**6.22**	**-13.3**	-1.42	2.17	**16.6**	**70.6**	**22.9**	**21.4**
BaP	**-7.85**	ND	**5.47**	**7.22**	0.26	**3.03**	**13.3**	-0.37	**8.19**	**-8.81**	**-17.0**	-1.04	1.77	**-18.3**	**4.50**	0.68
IDP	**-6.14**	ND	**7.32**	**10.59**	1.41	**14.74**	**36.89**	0.50	**7.31**	**-5.35**	-0.68	**3.06**	**3.48**	**7.00**	**17.6**	**9.28**
DBA	**-2.97**	ND	**6.27**	**5.22**	0.80	0.30	ND	**-14.9**	**7.39**	**-3.69**	-1.85	1.25	**9.75**	**13.5**	**14.2**	**52.3**
BgP	**-5.51**	ND	**7.59**	**7.81**	1.27	1.22	2.73	-0.94	**7.80**	**-6.97**	**-5.30**	0.79	**3.09**	**3.02**	**6.56**	**3.14**

ND = no data for an individual PAH for at least one size fraction

The figures in bold represent the statistically significant values (above $t_{critical}$) (95% confidence interval), of comparisons between individual PAH concentrations in soil fractions A and B.

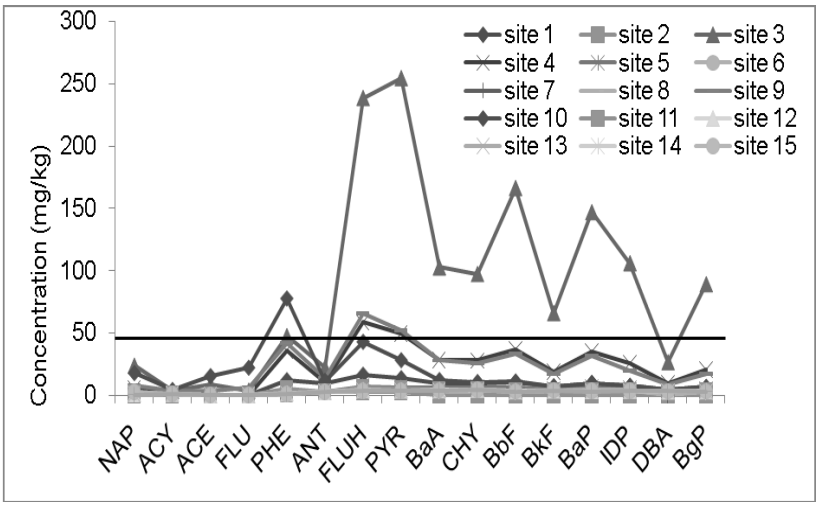
Soil fraction A = < 250 µm and soil fraction B = > 250 µm < 2 mm

Conversely, 31.1% of the mean individual PAH concentrations showed no significant difference (95% confidence interval) between soil fractions A and B.

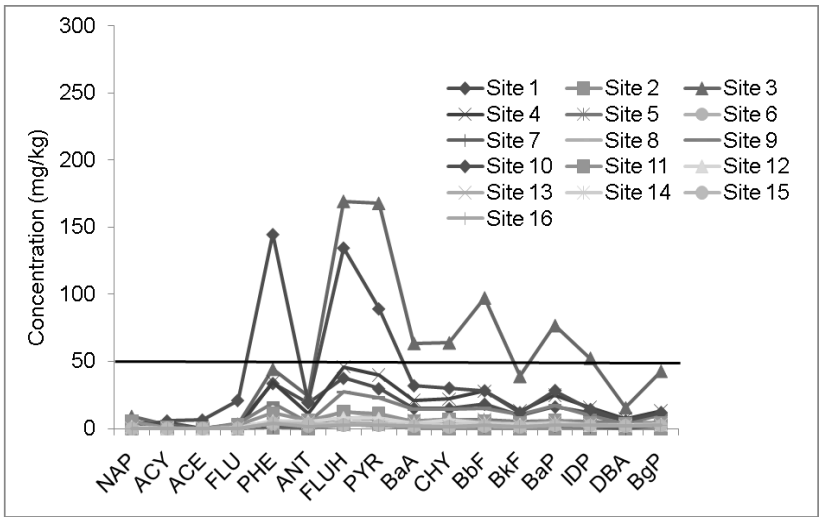
The results for both total and individual PAH concentrations indicated that PAHs were present in greater concentrations in soil fraction A than in soil fraction B. This may be because PAHs are more readily adsorbed with finer particles in the soil such as clay minerals and fine silt (Amellal *et al.*). However, while some workers have also found higher concentrations of PAHs in the smaller particle size fraction (150 – 250 μm) (Ahrens *et al.*, 2004) others have found higher concentrations of PAHs in the greater particle size fraction i.e. 250 – 500 μm (Li *et al.*, 2010). Based on our results, however, the higher concentration of PAHs in soil fraction A is important in contaminated land studies. This is because fraction A (< 250 μm) corresponds to the particle size thought to be the most important in terms of human contact with soils and potential health risk (Bornschein *et al.*, 1987; Rodriguez *et al.*, 1999; US Environmental Protection Agency, 2000).

Individual PAH concentrations in each of the soil samples are shown in Figure 5.3 (a) and (b) for soil fractions A and B, respectively. In soil fractions A and B, the majority of samples had individual PAH concentrations < 50 mg/kg. The commonly used Dutch intervention level (40 mg/kg) is often used as a guide to total PAH in soils (VROM, 2000). However, it may not be appreciated that it is based on the sum of ten individual PAH. Therefore, in this work, it was considered appropriate to select a higher value (50 mg/kg) as the boundary between high and low individual PAH concentrations, based on the determination of 16 compounds. Based on this value of 50 mg/kg it is noted that the soil from sampling site 3, irrespective of soil particle size, contained the highest concentrations of individual PAHs. Other exceptions included soils 4 and 9 for which elevated concentrations of fluoranthene and pyrene were identified, and for site 10 high levels were found for phenanthrene, all of them in soil fraction A (Figure 5.3 a). Soil from sampling site 10 also contained elevated concentrations of phenanthrene, fluoranthene and pyrene - in fraction B (Figure 5.3 b).

(a)



(b)



Soil fraction A = < 250 µm and soil fraction B = > 250 µm < 2 mm

See Table 1 for explanation of PAH abbreviations

Figure 5.3: The individual PAH concentrations of (a) soil fraction A and (b) soil fraction B from the former St. Anthony's Tar Works study area.

5.3.4 Distribution and sources of PAHs across the St Anthony's Tar Works study area

The results for total and individual PAH concentrations in the soil samples demonstrated that highest values were reported at sampling sites 1, 3, 4, 9 and 10 (Figure 5.1). The most polluted sites were close to where the former factory was located (sites 1 and 3; Figure 5.1) (P Hartley, Newcastle City Council, Personal

Communication, 2009). Sampling site 2 which was proximal to sites 1 and 3 had a lower soil PAH concentration (individual PAH concentrations ranged from 0 to 3.0 mg/kg). This may be because site 2 corresponded to the location of the former factory, which rested on an impervious floor and this may have protected the sampling site from pollution spillage. Site 4 was located adjacent to a vertical conduit which might have acted as a storage area for the factory; hence the higher PAH concentrations in this soil. Sample sites 7 – 15 were located on the foreshore of the river and as such were subject to twice-daily tidal washing in the River Tyne estuary. This may explain the generally lower PAH concentrations reported in these soils. The exceptions to this trend were soils from sampling sites 9 and 10, which contained higher PAH concentrations. This probably reflects their location directly down-slope of any sub-surface run-off from the former factory (Figure 5.1).

The results for soil individual PAH concentrations demonstrated that higher molecular weight PAHs (from 3 to 6 rings) were recovered in greater concentrations compared to lower molecular weight PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene) across the study area (Figures 5.2 and 5.3). Specifically, fluoranthene and pyrene were recovered from the soils in significantly higher concentrations than the rest of the PAHs. It is possible that lower molecular weight PAHs could simply have evaporated from the study area over time due to their high volatility. Anecdotal evidence for this process was noted during sample collection from sites 7 – 12 (Figure 5.1) at which a strong hydrocarbon odour was evident. As outlined in the methodology and analytical figures of merit sections of this paper, it should also be borne in mind that lower molecular weight PAHs may have been lost in the sample processing / post extraction solvent evaporation process prior to GC-MS analysis (Dean, 2003; Lorenzi *et al.*, 2008).

However, despite these possible volatilisation processes, comparing the results of the present work with other studies into PAH distributions in anthropogenically contaminated soils, it is apparent that the trends are very similar. Generally the lower

molecular weight PAHs (e.g. naphthalene, acenaphthylene, acenaphthene) are the least recovered, the medium molecular weight PAHs (e.g. fluoranthene and pyrene) show the greatest recoveries, and finally the remaining PAHs, which include mainly high molecular weight compounds, are recovered in moderately elevated concentrations (Berset *et al.*, 1999; Trapido, 1999; Ong *et al.*, 2003; Motelay-Massei *et al.*, 2004; Nadal *et al.*, 2004; Graham *et al.*, 2006; Morillo *et al.*, 2007). It has been well documented in the literature that PAHs recovered from sites that are typical of anthropogenic (pyrogenic) sources tend to have high molecular weights as opposed to petrogenic sources which are typically characterised by the lower molecular weight PAHs (Li *et al.*, 2008). Therefore, it is most likely that the greater concentrations of higher molecular weight PAHs at the former site of the St Anthony's Tar Works are indicative of pyrogenic (anthropogenic) sources, given its industrial history.

5.3.5 Influence of organic matter and pH

The identification of the sources of PAH pollution seems to be an appropriate way to comprehend the variation in PAH distribution in this particular site. Some studies have demonstrated that the content of organic matter or pH in a soil could potentially involve interaction with compounds that may retain them in the matrix (Chiou *et al.*, 1979; Means *et al.*, 1980; Chiou *et al.*, 1986; Calvet, 1989; Yin *et al.*, 1996). Therefore, an estimation of organic matter content and pH was realized on the different soils sample at two different particle sizes, compared to the distribution of PAHs on the site (Table 5.4 and 5.5). Firstly, variations in the content of organic matter between sampling site were very low, with values varying from 9.4 to 22.4 % LOI. . Consequently, the identification of trends was complicated. Moreover, the rare variations were showing contradictory trends. Indeed, low organic matter content was giving both high total PAH content and low total PAH content (9.4 % LOI giving 375 mg/kg and 11.0 % LOI giving 9.0 mg/kg). And in the same way high organic matter content was showing both low and high total PAH content (18.4 % LOI giving 1404 mg/kg and 22.4 % LOI giving 38.9 mg/kg).

Table 5.4: Comparison of loss of ignition (%LOI) and total PAH content in two different particle sizes of soil (< 250 µm and > 250 µm)

	Particle size < 250 µm		Particle size > 250 µm	
Soil sample site	**% LOI**	**Total PAH content (mg/kg)**	**% LOI**	**Total PAH content (mg/kg)**
1	11.1	123	12.1	234
2	11.0	9.0	11.2	6.6
3	18.4	1404	15.3	872
4	17.4	366	11.8	285
5	15.9	66.5	17.9	69.2
6	15.2	46.4	15.4	39.9
7	22.4	38.9	NA	NA
8	19.9	40.5	NA	NA
9	9.4	375	13.9	173
10	17.2	289	NA	NA
11	13.5	54.1	NA	NA
12	15.4	43.6	NA	NA
13	20.1	41.6	NA	NA
14	19.9	40.8	NA	NA
15	22.3	43.7	NA	NA
16	21.4	39.7	20.5	28.3

*NA= Non Available

Moreover, the differences in total PAH content between the two particle sizes did not show any associations with organic matter variations, as the variation in organic matter were not significant compared to the variations in total PAH content. It was concluded that the PAH distribution on this site was independent of organic matter and more linked to the type of locations where the samples were collected. The pH range of values was also contained in a narrow range between 6.51 and 8.72, and as with the comparison with organic matter content, the total PAH content was found to be completely independent from the variations in those pH values. pH variations between the two particles sizes were negligible, having no influence on the total PAH content differences observed previously (Table 5.5).

Table 5.5: Comparison of pH (calculated in water and $CaCl_2$) with the total PAH content of two different particle sizes (< 250 µm and > 250 µm)

	Particle size < 250 µm			Particle size > 250 µm		
Soil sample site	**pH (distilled water)**	**pH ($CaCl_2$)**	**Total PAHs content (mg/kg)**	**pH (distilled water)**	**pH ($CaCl_2$)**	**Total PAHs content (mg/kg)**
1	7.61	7.16	123	7.83	7.55	234
2	8.34	7.67	9.0	8.44	7.41	6.6
3	7.41	7.2	1404	7.11	6.97	872
4	7.75	7.17	366	8.23	7.09	285
5	7.81	6.81	66.5	7.73	6.81	69.2
6	7.45	6.57	46.4	7.75	6.49	39.9
7	6.58	6.61	38.9	NA	NA	23.6
8	6.51	6.52	40.5	NA	NA	65.8
9	8.14	7.48	375	8.72	7.96	173
10	7.09	6.89	289	NA	NA	585
11	7.04	6.82	54.1	NA	NA	88.7
12	6.84	6.74	43.6	NA	NA	59.0
13	6.87	6.75	41.6	NA	NA	30.6
14	6.91	6.73	40.8	NA	NA	38.8
15	6.86	6.62	43.7	NA	NA	28.4
16	6.53	5.86	39.7	6.85	5.76	28.3

5.4 Conclusion

The importance of determining PAHs associated with different soil particle size fractions has been highlighted in this work. The higher concentrations of PAHs in soil fraction A (< 250 µm particle size) are important to highlight as this soil fraction is most likely to be accidentally ingested by humans (Bornschein *et al.*, 1987; Rodriguez *et al.*, 1999; US Environmental Protection Agency, 2000) These findings have implications for the development of ongoing Soil Guideline Values for PAHs in relation to environmental human health risk, which are typically based on a < 2 mm soil size fraction only.

The distribution of individual PAH in soils across the former site of the St Anthony's Tar Works, coupled with the history of the site, indicate that the PAHs are most probably derived from pyrogenic (anthropogenic) sources. The dominance of higher

molecular weight PAHs across the site is consistent with trends reported in other anthropogenically polluted soils from around the world. The distribution of PAHs on the site was principally linked to the sample locations, related to the position where chemicals were produced in the former factory. The distribution of the PAHs on the site was shown to be independent from the organic matter and the pH content of those soils, in contradiction with observations made in the literature. The total PAH content variations between the two particles sizes was therefore not related to those parameters, and seems more likely to be due to other properties of the soils such as the surface area, which will increase at finer grain size, increasing sorption of PAHs. However, the notably high concentrations of soil PAHs determined at this site, compared to other contaminated locations reported in the literature, make it a prime target for further investigation/remediation given its proximity to a national cross-country pathway of historic importance (Hadrian's Wall walk) and a popular venue on the River Tyne foreshore for fishing.

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CHAPTER 6: Application of two versions of an *in vitro* gastrointestinal extraction to evaluate oral bioaccessibility of PAH in industrially contaminated soils

6.1 Introduction

As described in the previous chapter, the ingestion of a solid matrix depends on the potential adsorption against the skin, according to the size, type and the surface of the matrix particles (soil, dust and sediment). After being adsorbed the matrix will enter human digestion, and may cause harm via mobilization of pollutants. Then, small quantities of the solid matrix will be ingested with nutrients, releasing potential contaminants inside the gastrointestinal tract. A risk for human health will exist, as the contaminants can interact with the organs inside the gut. To assess the effects of the ingestion of pollutant via soils, *in vitro* methods have been introduced to estimate the bioaccessibility of soil contaminants, as an indicator of *in vivo* bioavailability (Scoof, 2004). Indeed*, in vitro* methods are being developed in order to reduce and replace human and animal testing, which involve financial and ethical issues (Environment Agency, 2007).

The Environmental Agency in England and Wales agrees that the development of an *in vitro* digestion test that is robust, simple and representative of reality would be a useful tool to assess human health risk from pollutants in environmental matrices, in commercial laboratories (Environment Agency, 2005). This simulated *in vitro* gastrointestinal test could be used in risk assessment if it is simple, comprehensive, precise, reproducible, interpretable and consistent (Gron *et al.*, 2003). Currently, physiologically-based extraction tests have only started to show good reproducibility (Versantvoort *et al.*, 2004; Oomen *et al.*, 2006) within and between laboratories; there is still need for certified reference materials concerning bioaccessibility testing, and there is still no agreed procedure that every laboratories could use for any matrix and any contaminants (Environment Agency, 2005). However, recent studies, developed by the Bioaccessibility Research Group of Europe (BARGE), demonstrated

satisfactory reproducibility for bioaccessibility testing for matrices such as food and soils containing contaminants, such as metals (Versantvoort *et al.*, 2004; Wragg *et al.*, 2009). The members of this group have also developed the Unified BARGE Method (Cave *et al.*, 2006), which is used in the present study, by comparing several physiologically-based extraction tests.

In order to represent more accurately and realistically human digestion of soil containing polycyclic aromatic hydrocarbons, some researchers have developed a fed version of the physiologically-based extraction test. Indeed, a child is thought to be in a fed state more than half of the time during a day, considering ingestion of snacks through the day (Oomen *et al.*, 2006). A Danish study (Gron *et al.*, 2007) and a Dutch study (Sips *et al.*, 2001; Versantvoort *et al.*, 2004), based on a report from the Dutch National Institute for Public Health and the Environment (RIVM), estimated PAHs bioaccessibilities from food and soils using fed and fasted gastrointestinal tests (Versantvoort *et al.*, 2004; Oomen *et al.*, 2006).

Moreover, the RIVM has largely contributed to the development of the UBM (Unified BARGE Method) (fasted state) (Cave *et al.*, 2006), and the fed version of this test, the FORES(h)t (Fed Organic Estimation human Simulation Test) (Cave *et al.*, 2010), used in the present study, with minor modifications, because of changes in secretion of enzymes and variations in pH, when food is ingested. In the case of the FORES(h)t method, developed and named by members of BARGE (Cave *et al.*, 2010) in England, the food supplement in the test was composed of pure sunflower oil and an organic creamy porridge infant food and was based on the macronutrients composition of the average diet of a 4-6 years old child in the UK (Gregory *et al.*, 2000). The amount of soil added during the process was based on the fact that children ingest involuntarily 100 mg of soil per day via hand-to-mouth behaviour (Oomen *et al.*, 2006; U.S Environmental Protection Agency, 2008). According to the RIVM and the US EPA this amount of daily involuntarily ingested soil considers a child aged between 1 and 6 years old (Oomen *et al.*, 2006; U.S Environmental Protection

Agency, 2008). Based on these assumptions, 0.3 g of soil was used with those models, in order to obtain a soil-to-solution ratio closer to the fluid proportion of the gastrointestinal tract of a child (Oomen *et al.*, 2006). The composition of the gastrointestinal fluids is based also on the human physiology of the gastrointestinal tract (Oomen *et al.*, 2003). The main reason for adding food in such systems is to mimic more realistically the human digestion. Consequences of adding food in an *in vitro* gastrointestinal test were described as an increase of the mobilization of hydrophobic PAHs from the matrix, inside the human gastrointestinal fluids. This was demonstrated to be partly due to the changes in the gastrointestinal fluids composition between a fasted and a fed state. Indeed, parameters that can alter mobilization of PAHs can be the amount of bile salts (Friedman *et al.*, 1980; Fries, 1985; Feroci *et al.*, 1995; Hack *et al.*, 1996; Charman *et al.*, 1997; Luner, 2000; Oomen *et al.*, 2000; Holman *et al.*, 2002; Pu *et al.*, 2004; Van de Wiele *et al.*, 2004), the soil-to-solution ratio (Van de Wiele *et al.*, 2004), the amount of food (Fries *et al.*, 1989; Hack *et al.*, 1996; Van Schooten *et al.*, 1997; Shargel *et al.*, 1999; Roos *et al.*, 2000; Wittsiepe *et al.*, 2001; Pu *et al.*, 2004), the quantity of mucine (Hack *et al.*, 1996), the chlorine content (Van den Berg *et al.*, 1985 ; Geyer *et al.*, 1987; Olling *et al.*, 1990; Mc Lahan, 1993; Wittsiepe *et al.*, 2001; Schwarzenbach *et al.*, 2003; Pu *et al.*, 2006), the ring number (NEPI, 2000; Tang *et al.*, 2006), the organic matter (Chiou *et al.*, 1986; Calvet, 1989; Yin *et al.*, 1996; Kogel-Knabner *et al.*, 2000; Schwarzenbach *et al.*, 2003; Pu *et al.*, 2004; Van de Wiele *et al.*, 2004), the solubility of individual PAHs in water (Mackay, 2001), and the physicochemical properties of the soils (Chung *et al.*, 1998; Ake *et al.*, 2001; Pu *et al.*, 2004; Pu *et al.,* 2006)

Furthermore, the addition of food constituents in the *in vitro* gastrointestinal test will considerably modify the related analytical methods used to isolate PAHs from the resulting simulated gastrointestinal digests. A complex matrix consisting of food constituents, biological fluids and soil particles will require further steps of extraction and purification, involving for example saponification (Grimmer *et al.*, 1975;

Eschenbach *et al.*, 1994; Hartman, 1996; Kelly *et al.*, 2000; Northcott *et al.*, 2001; Kishikawa *et al.*, 2003; Pena *et al.*, 2007; Itoh *et al.*, 2008), than for a fasted version of the *in vitro* digestion test. Food residues and alkaline substances resulting from saponification need to be removed or cleaned-up before injection on a GC-MS, so as to avoid any spoiling of instrumental parts such as the column, the ion trap (or quadrupole) and the filament. The FORES(h)t method has already been tested as precise, accurate and robust using HPLC-FL as the analysis method (Cave *et al.*, 2010).

A recent survey has shown that 70 % of scientists and policy makers, working in this area of research, think that the use of bioaccessibility testing is a powerful tool that refines risk assessment and facilitates sustainable land management (Latawiec *et al.*, 2010). In the UK, the Soil Guidelines Values are based on the Contaminated Land Exposure Assessment model, which also describes how to estimate human health risk from pollutants in environmental matrices. The model was developed in order to compare predicted contamination exposure levels with known toxicological or Health Criteria Values (HCVs). A HCV represents the exposure level below which, there should be minimal or no risk to human health (Environment Agency, 2007). Those values are used to derive a Soil Guideline Value (SGV) which is dependent on the site and the contaminant considered (Environment Agency, 2007). Derivation of SGVs using the CLEA model assumes that the contaminant is released from the soil and is taken up by the human body to the same extent as the model which has been used to determine the HCV (for that contaminant) (Environment Agency, 2005). This assumption may, of course, not be true as the HCVs may have been determined using non-human participants and more soluble forms of the contaminant (Environment Agency, 2005). Consequently, basing the human health risk of harmful compounds from contaminated land on SGVs implies that the entire pollutant is entering the bloodstream. This statement does not take into consideration the

insolubility of the contaminant and its potential weathering and chemical bonding within the soil (Environment Agency, 2005).

Another way to estimate the risk to humans from pollutants is to compare the mean daily intake with the amount of PAH ingested (µg) through involuntary ingestion (100 mg/day), soil-pica (50 g/day) and geophagy behaviour (1g/day) (U.S Environmental Protection Agency, 2008). These calculated values give an estimation of the risk from pollutants when soils are ingested. This calculation of the PAH intake will describe how they can interact and be mobilized in the digestive tract. However, this calculation is still based on the total pollutant content obtained in the environmental matrix, which is not as realistic as the determination of bioaccessibilities, considering the ingestion exposure pathway. Indeed, the bioaccessible concentration can be calculated according to the bioaccessible fraction and be compared with the mean daily intake. There are no threshold values concerning the bioaccessibility (%), as values may vary from 0 to 100 %, but it can be suspected that the risk will increase as the bioaccessibility and bioavailability is increasing.

This chapter will focus (1) on the application of the Unified BARGE Method, by evaluating the analytical method through a spiking procedure (insert a known amount at the beginning of the procedure and observe response at the end of the process), and then a comparison of total PAH content, residual digests and PAH bioaccessible fractions. The chapter will then deal with (2) the application of the FORES(h)t method, by evaluating the analytical method through a spiking procedure, and then comparing total PAH content, residual digests and PAH bioaccessible fractions. Then, (3) a comparison will be made between PAH bioaccessibilities and total PAH content in two different soil locations, evaluating any parameters that could influence distribution of PAHs. An interlaboratory comparison (4) will be realized for the application of the FORES(h)t method in two different laboratories. Finally (5), a risk assessment evaluation will be established according to the bioaccessible fractions observed in different site locations.

6.2 Experiment

6.2.1 The study site

The study site (St Anthony's Tar works), sample collection strategy, soil pre-treatment procedure, storage, extraction, as well as the apparatus needed for the experiment has already been described in chapter 4 and 5. The other soil samples used in this study were supplied by the British Geological Survey, from disused gas work sites within the UK (Cave *et al.*, 2010). They were firstly freeze-dried and sieved below 250 µm (Cave *et al.*, 2010) as for the soils collected from the St Anthony's Tar works, considering this fraction size as potentially more easily ingested through hand-to-mouth contact with soil (Bornschein *et al.*, 1987; Rodriguez *et al.*, 1999; US Environmental Protection Agency, 2000)

6.2.2 Apparatus for analysis of total PAHs content

In order to obtain the total PAHs concentration in the soils and in the residues after the simulated *in vitro* gastrointestinal tests, the analytical method *in-situ* PFE-GC-MS developed in chapter 4 for the analysis of PAHs in soils was used. The chemicals, apparatus, and instrumentation related to that method were also described in chapter 4.

Two different GC-MS systems were used, one with the Unified BARGE Method (ion trap), and the other with the FORES(h)t (quadrupole). Two different types of mass spectrometers were used because of the FORES(h)t method involving complex samples containing food components. Using a quadrupole instead of an ion trap will increase instrument robustness and decrease sensitivity. The first GC-MS instrument, used to analyse the extract from the UBM, was a Trace GC coupled with a Polaris Q (ion trap) mass spectrometer (Thermo Scientific, UK) and a Triplus auto sampler injector. The second GC-MS, used to analyse the extract resulting from the FORES(h)t, was a FOCUS GC coupled with a DSQ (single quadrupole) and an Autosampler AS 3000 (Thermo Scientific, UK). Identical temperature programs and

capillary columns were used in both instruments. The injector was used in Split according to the SSL mode. A five point calibration curve was used for quantitation on the GC-MS using 4, 4' difluorobiphenyl (10 mg/kg) as an internal standard. A sonicator (Bransonic Ultrasonic Cleaner 2200) was used to sonicate PAHs standards solutions.

6.2.3 Unified BARGE Method: Chemicals and laboratory equipment

Table 6.1: Reagents used in the Unified BARGE Method and FORES(h)t method with their respective supplier and supplier location

Reagent	Supplier	Location
NaCl	Merck	Darmstadt, Germany
KSCN	Merck	Darmstadt, Germany
KCl	Merck	Darmstadt, Germany
$CaCl_2.2H_2O$	Merck	Darmstadt, Germany
NH_4Cl	Merck	Darmstadt, Germany
$NaHCO_3$	Merck	Darmstadt, Germany
$MgCl_2.6H_2O$	Merck	Darmstadt, Germany
NaOH	Merck	Darmstadt, Germany
HCl	Merck	Darmstadt, Germany
Urea	Merck	Darmstadt, Germany
Anhydrous D+Glucose	Merck	Darmstadt, Germany
D-Glucosaminehydrochloride	Merck	Darmstadt, Germany
Pepsin (pig)	Merck	Darmstadt, Germany
Bovine Serum Albumin (BSA)	Merck	Darmstadt, Germany
Pancreatin (pig)	Merck	Darmstadt, Germany
Mucin (pig)	Merck	Darmstadt, Germany
Uric acid	Merck-Prolabo	Leuven, Belgium
α-amylase (bacillus species)	Sigma Aldrich	Dorset, UK
Lipase (pig)	Sigma Aldrich	Dorset, UK
Bile salts (bovine)	Sigma Aldrich	Dorset, UK
D-Glucoronic acid	Sigma Aldrich	Dorset, UK
NaH_2PO_4	Mallinckrodt, Baker	Devender, Holland
KH_2PO_4	Mallinckrodt, Baker	Devender, Holland
Na_2SO_4	May and Baker Ltd	England
Sunflower oil	Marks and Spencer	Chester, UK
HIPP creamy porridge	HIPP Ltd	Berkshire, UK

The UBM procedure was done using an end-over-end shaker (Stuart® rotator SB3) in an oven fixed at the temperature of 37 ± 2 °C, a water bath (Grant OLS 200), a pH meter (3020 JENWAY supplied by S.H Scientific), and a centrifuge (Centaur 2 Sanyo;

MSE, Scientific Laboratory Supplies). To avoid contact of PAHs with plastic we used 1 L and 500 ml Duran glass vessels to prepare the biological fluids. The sample preparation of the soils was done in red-top centrifuge tubes (Sarstedt Ltd, Leicester) suitable for further shaking and centrifugation. The oven used to heat samples at 100 ºC for 1 hour during the saponification was from Sanyo Electric Ltd, Japan.

6.2.4 Unified BARGE Method: Preparation of the gastrointestinal fluids

The physiologically-based extraction test from the present study was based on the Unified BARGE method (Cave *et al.*, 2006)**.** Essentially, simulated saliva fluid was prepared by first adding 145 mg of amylase, 50.0 mg mucin and 15.0 mg uric acid to a 1 litre Duran bottle. Then, separately, 896 mg of KCl, 888 mg NaH_2PO_4, 200 mg KSCN, 570 mg Na_2SO_4, 298 mg NaCl and 1.80 mL of 1.0 M HCl were added into a 500 mL volume container and made up to the mark with water into a second 500 mL volume container, 200 mg of urea was added and made up to the mark with water. Then, simultaneously the 500 mL of inorganic and 500 mL of organic saliva components were poured into the 1 litre Duran bottle. The entire content of the bottle was shaken thoroughly. The pH of this solution was measured (gastric simulated saliva fluid). The pH needed to be within the range 6.5 ± 0.5 (if necessary, pH was adjusted by adding either 1.0 M NaOH or 37 % HCl v:v).

Simulated gastric fluid was prepared by first adding 1000 mg of bovine serum albumin, 3000 mg mucin and 1000 mg pepsin to a 1 litre Duran bottle. Then, separately, 824 mg of KCl, 266 mg NaH_2PO_4, 400 mg $CaCl_2$, 306 mg NH_4Cl, 2752 mg NaCl and 8.30 mL of 37 % HCl v:v were added into a 500 mL volume container and made up to the mark with water. Into a second 500 mL volume container, 650 mg glucose, 20.0 mg glucuronic acid, 85.0 mg urea and 330 mg glucosamine hydrochloride were added and made up to the mark with water. Then, simultaneously the 500 mL of inorganic and 500 mL of organic components were poured into the 1 litre Duran bottle. The entire contents of the bottle were shaken thoroughly. pH of this solution was measured (gastric simulated fluid). The pH needed to be within the range

0.9-1.0. When necessary, pH was adjusted to this range (0.9-1.0) by adding either 1.0 M NaOH or 37 % HCl. The combination of mixed saliva fluid (1 mL) and gastric fluid (1.5 mL) was checked for pH in the range 1.2 – 1.4. If the combined mixture was not within this range it was necessary to adjust the pH of the gastric fluid by adding either 1.0 M NaOH or 37 % HCl. The combination of mixed saliva fluid (1 mL) and gastric fluid (1.5 mL) was again checked for a pH in the range 1.2 – 1.4.

Simulated duodenal fluid was prepared by first adding 200 mg of $CaCl_2$, 1000 mg bovine serum albumin, 3000 mg pancreatin and 500 mg lipase to a 1 litre Duran bottle. Then, separately, 564 mg of KCl, 80 mg KH_2PO_4, 50.0 mg $MgCl_2$, 5607 mg $NaHCO_3$, 7012 mg NaCl and 180 µL of 37 % HCl were added into a 500 mL volume container and made up to the mark with water. Into a second 500 mL volume container, 100 mg urea were added and made up to the mark with water. Then, simultaneously the 500 mL of inorganic and 500 mL of organic duodenal components were poured into the 1 litre Duran bottle. The entire contents of the bottle were shaken thoroughly and pH of this solution was measured (simulated duodenal fluid). The pH needed to be within the range 7.4 ± 0.2. So when necessary, the pH of the duodenal fluid was adjusted by adding either 1.0 M NaOH or 37 % HCl v:v.

Simulated bile fluid was prepared by first adding 222 mg of $CaCl_2$, 1800 mg bovine serum albumin and 6000 mg bile to a 1 litre Duran bottle. Then, separately, 376 mg of KCl, 5785 mg $NaHCO_3$, 5259 mg NaCl and 180 µL of 37 % HCl v:v were added into a 500 mL volume container and made up to the mark with water. Into a second 500 mL volume container 250 mg urea was added and made up to the mark with water. Then, simultaneously the 500 mL of inorganic and 500 mL of organic bile components were added into the 1 litre Duran bottle. The entire contents of the bottle were shaken thoroughly. The solution was left to stand for approximately 1 hour, at room temperature, to allow for complete dissolution of solid reagents. The pH of this solution was measured (simulated bile fluid). The pH needed to be within the range

8.0 ± 0.2. When necessary, the pH of the duodenal fluid was adjusted by adding either 1.0 M NaOH or 37 % HCl v:v. The combination of 1.0 mL saliva fluid, 1.5 mL gastric fluid, 3.0 mL duodenal fluid and 1.0 mL bile fluid was checked for a pH around 6.3 ± 0.5. If the combined mixture was not within this range it was necessary to adjust the pH of the duodenal fluid by adding either 1.0 M NaOH or 37% HCl v:v. The combination of 1.0 mL saliva fluid, 1.5 mL gastric fluid, 3.0 mL duodenal fluid and 1.0 mL bile fluid was again checked for a pH at 6.3 ± 0.5. All extraction fluids were prepared the day before applying the *in vitro* gastro-intestinal test, to let all the reagents dissolve overnight (stored at < 8 °C).

6.2.5 Unified BARGE Method: Extraction of samples

An accurately weighed soil sample (0.3 g) was placed into a 50 mL screw cap Sarstedt tube and treated with 9 mL of simulated saliva fluid by manually shaking the mixture in the screw-cap vessel. Then, after 5 – 15 minutes, 13.5 mL of simulated gastric fluid was added. The mixture was then shaken on an end-over-end shaker maintained at 37 ± 2 °C for 1 hour. Then, the pH of the soil suspensions was checked; the pH needed to be within the range 1.2 -1.7. Then, 27.0 mL of simulated duodenal fluid and 9.0 mL of simulated bile fluid were added by manually shaking the mixture in the screw-cap vessel. The pH of the resultant suspension was adjusted to 6.3 ± 0.5, by the drop wise addition of 37 % HCl v:v, 1 M or 10 M NaOH, as required. The mixture was again shaken on an end-over-end shaker maintained at 37 ± 2 °C for 4 h. Then, the soil suspension was removed and the pH of the soil suspension was recorded. The pH needed to be within the range 6.3 ± 0.5. The soil suspension was then centrifuged at 3000 g (6424.5 rpm) for 5 min. At the end of the process the liquid phase is taken with a micropipette (eppendorf) into the SPE cartridge until no more liquid can be taken. Then, each aqueous solution is passed through the cartridges positioned on the vacuum manifold of the SPE system (after conditioning the cartridges; following the SPE protocol). When all the aqueous phase is removed with a micropipette, and added on SPE cartridges, the sorbents were washed and PAHs

were eluted with dichloromethane (SPE protocol). Soil residues were dried during one or two days in Sartstedt tubes (50 ml). Then, after this period, the soils were delicately removed from the tube into small containers to be accurately re-weighed. The soils were again weighed to check any losses and the *in-situ* PFE procedure was again repeated.

6.2.6 Unified BARGE Method: spiking procedures

6.2.6.1 Liquid-liquid extraction

The spiking procedure was realized with water and the gastrointestinal juices to know the precision and accuracy of the results obtained by the operator on this specific method, and to check the potential influence of the gastro-intestinal fluids on PAHs recoveries. Firstly, an aliquot (10 ml) of reagent water, gastric or intestinal solution was taken. Thanks to a separatory funnel (50 ml) the aliquot of reagent water or UBM aqueous solution extract was added. Dichloromethane (5 ml) was added in the separate funnel and shaken for two minutes with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers was more than one third the volumes of the solvent layers, it was necessary to use mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of emulsion through glass wool, addition of salts and centrifugation. The dichloromethane extract was collected in an Erlenmeyer flask and then transferred into a volumetric flask. A second aliquot of dichloromethane (5 ml) was added to the sample bottle and into the separatory funnel so the extraction procedure was repeated a second time, then a third time collecting in the same Erlenmeyer flask (U.S. Environmental Protection Agency, July 1990). In order to observe recoveries of PAHs after extraction, and therefore the efficiency of the method, the solution was spiked at the beginning with 50 µl (2000 µg/ml standard PAHs solution) in 5 ml water sample. 40 µl of the internal standard solution (1000

µg/ml) was added at the end in the 20 ml volumetric flask and completed with solvent until graduation mark. At the end the PAHs standard concentration was 5 µg/ml and the internal standard concentration 2 µg/ml.

6.2.6.2 Solid Phase Extraction

As part of the spiking procedure with SPE, three types of sorbents were used: C2, C8 and C18. The same procedure was followed for the different types of sorbent as presented just below.

Conditioning Part: 2 X 10 ml of dichloromethane were passed through the SPE cartridge. The cartridge was let to drain under vacuum after each wash. Then 2 X 10 ml of methanol were passed through it and let drain after each wash. 2 X 10 ml of reagent water were passed through the cartridge. The first 10 ml portion was allowed to wash through and the cartridge was let to drain until dry. The next wash 10 ml portion was passing through it, keeping the cartridge wet and keeping the water level just above the sorbent of cartridge.

Loading part: 10 ml of the water sample were added keeping a very slow rate (1-2 drops per second). This step is very crucial because the compounds will start to be retained by the sorbent.

Wash part: 10 ml of distilled water were loaded through the sorbent. Vacuum was drawing through the cartridge for an additional 10 minutes to dry the cartridge. Then the vacuum was released and the sample waste discarded.

Eluting part: 2 X 5 ml of dichloromethane were passed through the cartridge and vessels were placed inside the vacuum manifold to collect the solvent. The container of the water sample was washed with 2 ml dichloromethane or more and added to the cartridge extract. This step was done at a very slow flow rate (1-2 drops per second) to get all the compounds from the cartridge (U.S. Environmental Protection Agency, July 1990).

Furthermore all glass surfaces coming in contact with the aqueous sample were washed with dichloromethane and added to the column eluate. In order to observe recoveries of PAHs and method performance after extraction, the solution was spiked inside the liquid in the vessel, to avoid any PAHs losses, at the beginning with 50 µl (2000 µg/ml standard PAHs solution) in 10 ml water sample. 40 µl of the internal standard solution (1000 µg/ml) was added at the end in the 20 ml volumetric flask and completed with solvent until mark. PAHs standards concentration was 5 µg/ml and the internal standard concentration 2 µg/ml.

6.2.6.3 Solid Phase Micro-Extraction

Firstly, the fibre was conditioned in the GC injection port at 250 ºC for one hour. Several fibre blanks were run to ensure the fibres were fully conditioned and that no interferences from the fibres were present in GC chromatograms. The fibre was placed inside a 10 ml aqueous solution with a holder allowing the fibre to go into the solution. SPME calibration curves involve the preparation of several standard solutions in a sample matrix so as to obtain the relationship between the peak responses and the targeted standard concentrations. Then, the concentrations of the target analyte in a sample can be calculated with the equation of the calibration curve (Ouyang et al., 2008). The solution was stirred during a fixed time (15, 30, 45, 60, 75, 90, 105, 120 min).

Finally, the fibre was directly inserted into the injection port of the GC-MS at the appropriate needle depth by adjusting the needle guide. The compounds will be desorbed at high temperature in the GC injection port. The amount of PAHs adsorbed against the fiber, as a function of stirring time, was controlled in order to know the best extraction time to recover them. To clean the PDMS fibres between injections they were immersed for 30 minutes in water soluble organic solvents such as methanol, acetonitrile or ethanol. In this case, the addition of water helps reduce swelling. The PAHs standard concentrations were established from 10 to 200 ng/ml.

6.2.6.4 Stir-Bar Sorptive Extraction

First the stir bar was conditioned as follow: it was placed into a vial containing 1 ml of a mixture of dichloromethane and methanol (1:1, v:v), and treated for 5 minutes with sonication. Then, the solvent mixture was rejected and the procedure repeated three times. The stir bar was dried in a desiccator at room temperature and heated for 90 minutes at 280 °C with a nitrogen stream of about 100 ml/min. Then the stir bar was placed into a glass vial having screw caps (for example 10 ml water sample in a 10 ml glass vial). The extraction was performed during 30 to 240 minutes with a stirring speed of 750 to 1000 rpm at room temperature (25 °C). After extraction, the stir bar was removed with clean tweezers and dried with a lint-free tissue. Then, for liquid desorption, the stir bar was placed into the insert (250 µl glass flat bottom) of a 2 ml vial. The insert was filled with 150 µl acetonitrile ensuring total immersion. Solvent back extraction was performed using ultrasonic treatment for 15 minutes at constant temperature: 25 °C (to obtain an efficient stripping) to allow desorption of the PAHs. After desorption the stir bar was removed by means of a magnetic rod, and the vial was placed into the auto sampler of the GC/MS. In order to observe recoveries of PAHs and method efficiency after extraction, the solution was spiked inside the liquid in the vessel, to avoid any PAHs losses, at the beginning with 50 µl (20 µg/ml standard PAHs solution) in 10 ml water sample. 50 µl of the internal standard solution (40 µg/ml) was added to the 200 µl solution in the 250 µl insert inside the vial. This will result on an internal standard concentration of 10 µg/ml and a PAHs standard concentration of 5 µg/ml. PAHs concentrations were observed according to variations in stirring time with the stirring speed kept constant and temperature let ambient (Garcia-Falcon et al., 2004).

6.2.6.5 Micro Extraction by Packed Sorbent

The system was totally automated, using the Triplus autosampler and the wash vial as sample solution. The packed syringe was conditioned first with methanol and then with water (50 µl) before being used for the first time. The water sample (50 µl each,

washing vial of the autosampler) was drawn through the syringe 30 times by the autosampler with a speed of 20 µl/s. The sample volume contained 10 ml of water and 5 µl of PAHs standard solution at 200 µg/ml. Consequently the PAHs concentration was 0.1 µg/ml in the water sample. The syringe was rinsed once by 50 µl of reagent water (sample vial). The analytes were then eluted with 30 µl dichloromethane (sample vial replaced by dichloromethane in another vial) directly into the GC injector, giving a final PAH concentration of 33.33 µg/ml.

6.2.7 FORES(h)t method

The FORES(h)t method is also a simulated in vitro gastrointestinal test which is based on the Unified Barge Method, with minor changes (Cave et al., 2010). Therefore, the same reagents used in the UBM were used in the FORES(h)t. The FORES(h)t method is the fed version of the UBM. Adding food in this test involves specific physiological changes such as the composition of the different fluids, the pH and the intestinal motility (Versantvoort et al., 2004). The pHs of the fluids were increased in comparison with the UBM (Cave et al., 2010). The amount of amylase was two times higher, and the quantity of mucin two times lower than in the UBM (saliva solution). The amount of mucin and pepsin in the gastric phase were multiplied by a factor of 3 for the former and by a factor of 2.5 for the latter. Pancreatin and lipase quantities were also increased by a factor of 3, for the composition of the duodenal fluid. Finally the amount of bile in the bile solution was increased by a factor of 5. pH values were also changed through the process. The pH of the saliva solution was augmented from 6.5 ± 0.5 to 6.8 ± 0.5, the gastric solution from 0.9-1 to 1.3 ± 0.5, the duodenal fluid from 7.4 ± 0.2 to 8.1 ± 0.2 and the bile fluid from 8.0 ± 0.2 to 8.2 ± 0.2 (Cave et al., 2010).

In order to realize the extraction, an accurately weighed sample (0.3 g) was placed into a 50 mL screw-cap Sarstedt tube with 0.813 g of HIPP organic creamy porridge (HIPP UK Ltd; Berkshire, UK), 2.45 ml of distilled water and 50 µl of pure sunflower oil

(Marks and Spencer; Chester, UK). Then, 4.5 mL of simulated saliva fluid was added via pipette and the solution was installed in the end-over-end shaker for 5 minutes with a speed of 30 rpm in an oven at 37 ± 2 °C. Then, 9 mL of simulated gastric fluid was added via pipette into the vessel. The tubes were capped and inserted in the end-over-end shaker inside an oven at 37 ± 2 °C for 2 hours. After the extraction, the vessels were removed from the extractor and the pH was controlled for each of the solutions. 9 ml of simulated duodenal fluid and 9.0 mL of simulated bile fluid were added via pipette to the vessels and the pHs of the solutions were measured before extraction in the oven. The pH was again checked after extraction of the solution during 2 hours, inside the oven at 37 ± 2 °C. The soil suspension was then centrifuged at 3000 g for 5 min.

After the centrifugation of the extract following the process, 1 ml of the liquid phase was transferred via glass pipette into a Hach chemical oxygen demand vial. The pipette was cleaned with 1 ml methanol into the tube, in order to get any PAHs possibly adsorbed on to the layer of the glass. Then, 3 ml of potassium hydroxide in methanol was added into the tube with a screw cap, and put into an oven for 1 hour at 100 °C. After cooling the solution, 5 ml of distilled water was added. Before loading the samples into the SPE polymeric cartridges (Waters OASIS HLB Plus Sep-Pak®) they were conditioned by adding 5 ml dichloromethane, 5 ml methanol and 2* 5 ml water. The samples were loaded through the cartridges at a flow rate of 1-2 ml/min. The cartridges were then washed by 5 * 2 ml of distilled water, and they were dried under maximum vacuum during 10 minutes. The clean-up step was realized by connecting the dried cartridge to a silica sorbent cartridge (Waters Plus Silica Sep-Pak®) in order to reverse the flow inside the cartridges compared to sample addition (Figure 6.1). In other terms, a backflush of the dried cartridges was realized into the silica cartridge. Finally, the cartridges were eluted by a solution of dichloromethane and tetrahydrofuran (1:1, v:v) at a slow flow rate, in 15 ml amber vials. Less than 10 ml of solvent was collected in each vial, and the solution was evaporated under a

gentle stream of nitrogen until dryness. The residue was collected with 1 ml or 100 µl of dichloromethane with 10 µl of an internal standard solution (respectively 1000 µg/ml or 100 µg/ml), according to the sensitivity obtained with the GC-MS for the analysis of the final solutions.

6.2.7.1 FORES(h)t method spiking procedure

To evaluate the performance of the analytical method following the FORES(h)t method, the soil was spiked at the beginning with 10 µl of a PAHs standard solution (2000 µg/ml). 5 replicates of each blanks and spikes were prepared so as to get accurate and precise results. The final solution was made to a volume of 1 ml with the internal standard (10 µl of a 1000 µg/ml) solution.

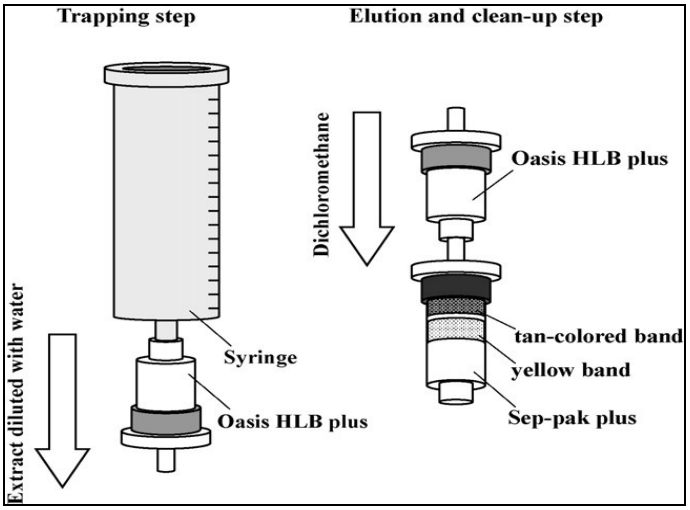


Figure 6.1: Solid phase extraction using polymeric and silica cartridges to isolate PAHs from gastrointestinal digests (Itoh *et al.*, 2008)

6.3 Results and Discussion

6.3.1 Quality control

The calibration curves were showing good linearity on both instruments with correlation coefficients above 0.995. Concentrations of standards were ranging from 0.1 µg/ml to 5 µg/ml. The first standard was chosen at a lower concentration than previous calibrations because of the low signal obtained after applications of the physiologically-based extraction tests, and purifications.

In order to lower the detection limit, pre-concentration was employed after the solid phase extraction by evaporating the solution until dryness under a gentle stream of nitrogen, and by completing with dichloromethane until 100 µl with the internal standard. Then, instead of a dilution factor around 100, the dilution factor was reduced to 10. Calibration curves and limits of detection, based on a signal-to-noise above 3, with the GC-DSQ are displayed on Table 6.1, the latter being converted to values in the FORES(h)t phase, and secondly converted to values in the residual digests and soil.

The calibration curves obtained with the GC-Polaris Q were obtained previously and are displayed in the Chapter 4. Solubility and partition coefficients (log K_{ow}) are also displayed because of the potential influence of the aqueous phase and the food matrix on the mobilization of PAHs, inside the digestive tract. Retention times of the PAHs between the chromatograms were slightly changed between the two instruments, but the PAHs peaks were sharp, showing good sensitivity and separation (Figure 6.2 and 6.3).

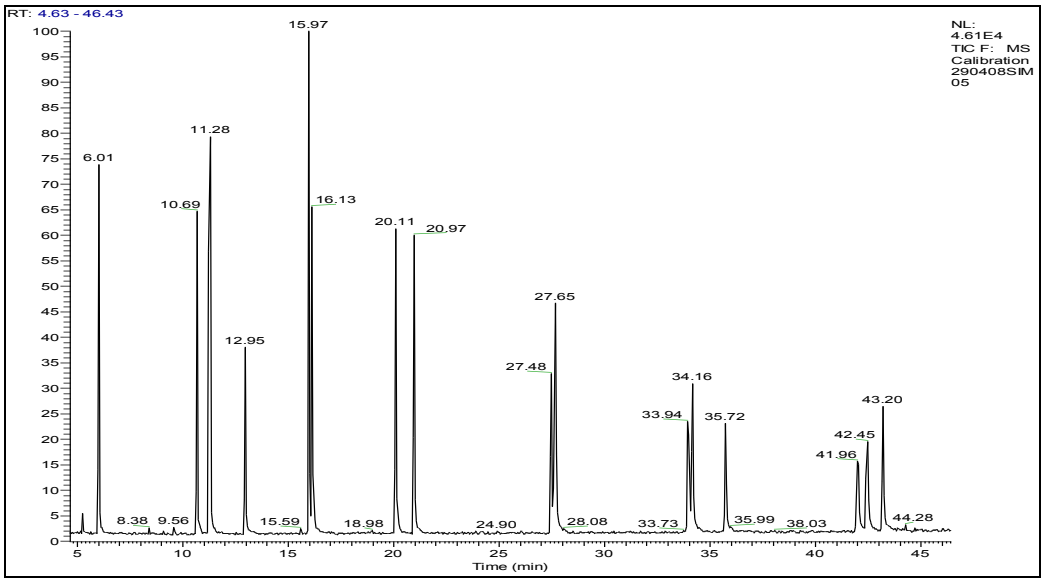
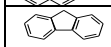
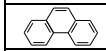
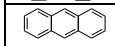
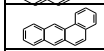
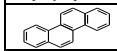
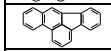
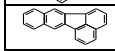
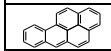
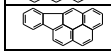


Figure 6.2: Chromatogram of a 5 µg/ml PAH standard solution using a Trace GC-Polaris Q (GC-MS) in SIM mode

Table 6.2: GC-MS calibration of PAHs based on a five point graph (0.1 - 5 µg/ml)

PAH Structure	Empirical Formula	PAHs	Number for PCA	MS Ion for Quantitation	LOD(mg/kg) in FORES(h)t solution	LOD (mg/kg) in soil and residue	Calibration Regression |y = mx + c|	Correlation Coefficient R^2	Solubility in water (µg/kg) at 25 °C[a]	Log K_{ow}[a]
	*C10H8*	NAP	1	*128*	1.15	1.44	*1.3313 X + 0.0865*	*0.9994*	31.7	3.35
	*C12H8*	ACY	2	*152*	1.10	1.38	*1.3079 X + 0.0981*	*0.9993*	3.93[b]	4.07[b]
	*C12H10*	ACE	3	*154*	1.44	1.80	*0.8795 X + 0.0880*	*0.9989*	3.42	3.92
	*C13H10*	FLU	4	*166*	1.77	2.21	*0.9513 X + 0.1655*	*0.9959*	1.98	4.18
	*C14H10*	PHE	5	*178*	1.43	1.79	*1.3456 X + 0.1703*	*0.9980*	1.29	4.57
	*C14H10*	ANT	6	*178*	1.35	1.69	*1.0494 X + 0.1035*	*0.9975*	4.5×10^{-2}	4.54
	*C16H10*	FLUH	7	*202*	1.94	2.43	*1.1869 X + 0.1665*	*0.9976*	2.6×10^{-2}	5.22
	*C16H10*	PYR	8	*202*	2.27	2.84	*1.2741 X + 0.1632*	*0.9971*	1.35×10^{-1}	5.18
	*C18H12*	BaA	9	*228*	1.60	2.00	*0.7502 X + 0.1146*	*0.9971*	5.7×10^{-3}	5.79
	*C18H12*	CHY	10	*228*	1.55	1.94	*0.9428 X + 0.1368*	*0.9971*	1.9×10^{-3}	5.98
	*C20H12*	BbF	11	*252*	1.55	1.94	*0.7314 X + 0.1042*	*0.9976*	1.4×10^{-2}	6.06
	*C20H12*	BkF	12	*252*	1.59	1.99	*0.9363 X + 0.1443*	*0.9964*	4.3×10^{-3}	6.06
	*C20H12*	BaP	13	*252*	1.11	1.39	*0.6183 X + 0.0613*	*0.9973*	3.8×10^{-3}	6.00
	*C22H12*	IDP	14	*276*	1.63	2.04	*0.5309 X + 0.0790*	*0.9959*	5.3×10^{-4}	6.40
	*C22H14*	DBA	15	*278*	1.67	2.09	*0.4932 X + 0.0782*	*0.9964*	4.0×10^{-4}	6.86
	*C22H12*	BgP	16	*276*	1.55	1.94	*0.6554 X + 0.0583*	*0.9979*	3.0×10^{-4}	7.10

[a] (Lu *et al.*, 2009)

[b] (Tang *et al.*, 2006)

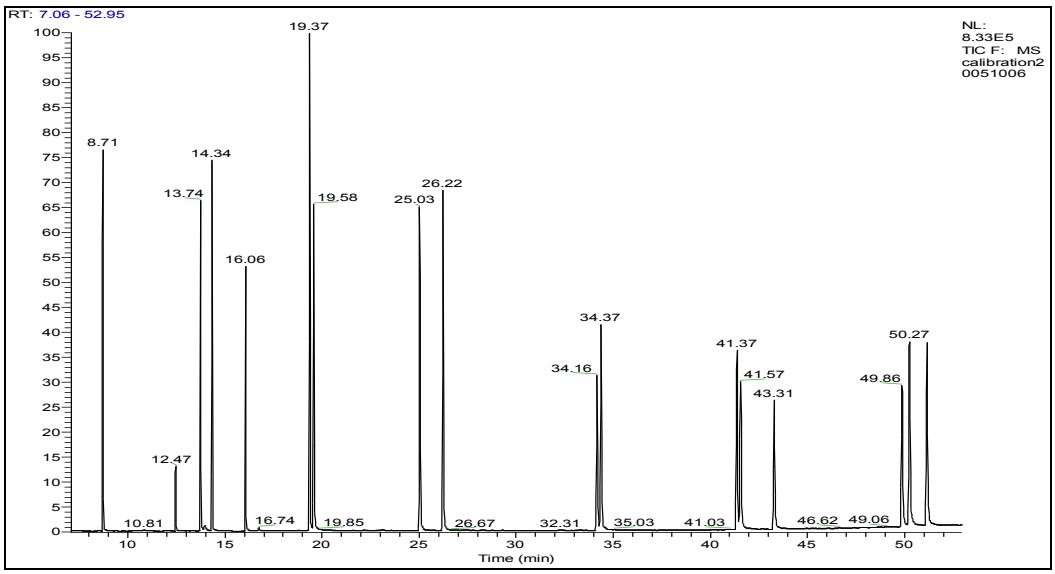


Figure 6.3: Chromatogram of a 5 µg/ml PAH standard solution using a Trace GC-DSQ (GC-MS) in SIM mode

The CRM analysis was done with two different masses, 0.3 g and 10 g. The lowest mass was considered as it was the mass used in the physiologically-based extraction models of the present study. Usually, a large amount of CRM was needed (e.g. 10 g, according to certificate recommendations) in order to compare confidently with the certificate values. The values obtained for 10 g of CRM were within the range of the certificate values, as shown in Table 6.2. They were all contained in the confidence interval, except one value, acenaphthylene, which was contained in the prediction interval. Concerning the extraction and analysis of 0.3 g of CRM, the values were lower than both the certificate and when using 10 g of CRM. The values were contained in the prediction interval, except two values, chrysene and benzo(a)pyrene which were slightly below the lower values of the prediction internal. Therefore, the values obtained with 0.3 g were lower than with 10 g. Using a F-test to compare values of PAHs concentrations for 0.3 g and 10 g, it shows that there was a statistically significant difference between the values, as the P value was below 0.05 (0.015) (Figure 6.4).

Table 6.3: Comparison of values (mg/kg) (CRM 123-100) resulting from the extraction of PAHs by *in-situ* PFE-GC-MS of 0.3 g and 10 g of certified reference material, with reference values (certificate value, confidence interval and prediction interval in mg/kg)

	CRM 123-100 (BNA's in soil)		CRM 123-100 reference values		
PAH	0.3 g Measured (+/- SD) n = 3 (mg/kg)	10 g Measured (+/- SD) n = 3 (mg/kg)	Certificate value (mg/kg)	Confidence Interval (mg/kg)	Prediction Interval (mg/kg)
Naphthalene	6.58 ± 0.63	8.07 ± 0.31	9.73	8.49-11.0	4.84-14.6
Acenaphthylene	3.39 ± 0.09	2.73 ± 0.10	7.24	5.75-8.73	1.37-13.1
Acenaphthene	5.49 ± 0.24	6.22 ± 0.23	7.52	6.20-8.84	2.31-12.7
Fluorene	5.83 ± 0.21	5.85 ± 0.07	6.88	5.91-7.85	3.05-10.7
Phenanthrene	5.06 ± 0.32	6.77 ± 0.21	7.94	6.96-8.92	4.07-11.8
Anthracene	4.50 ± 0.35	5.69 ± 0.14	6.94	5.90-7.98	2.83-11.1
Fluoranthene	5.20 ± 0.13	8.66 ± 0.51	9.31	8.08-10.5	4.44-14.2
Pyrene	4.22 ± 0.02	6.21 ± 0.14	6.75	5.79-7.71	2.98-10.5
Benzo(a)anthracene	4.55 ± 0.09	8.27 ± 0.34	8.38	7.24-9.52	3.87-12.9
Chrysene	6.01 ± 0.08	12.17 ± 0.40	11.3	10.0-12.6	6.23-16.4
Benzo(b)fluoranthene	NA*	NA	NA	NA	NA
Benzo(k)fluoranthene	NA	NA	NA	NA	NA
Benzo(a)pyrene	3.44 ± 0.21	7.35 ± 0.33	7.77	6.79-8.75	3.92-11.6
Indeno(1,2,3-cd)pyrene	NA	NA	NA	NA	NA
Dibenzo(a,h)anthracen	NA	NA	NA	NA	NA
Benzo(g,h,i)perylene	NA	NA	NA	NA	NA

*NA= non available

Ideally, the values should have been closer, considering homogeneity of the sample. But the soil-to-solution ratio may have influenced the potential release of compounds from the matrix.

It means that the values on the total PAHs concentrations of the samples were slightly underestimated, due to the use of only 0.3 g of soil samples. Consequently, the bioaccessible fraction may have been slightly overestimated as the PAHs concentrations were directly measured in the gastrointestinal digests, where no potential errors exist on those values. This study demonstrated again the need to obtain a certified reference material that allows measurement of the bioaccessible fraction for PAHs, or another certified reference material for soil samples that allows accurate measure of low-mass samples, such as 0.3 g, as a tool to realize the quality control on simulated *in vitro* gastrointestinal models.

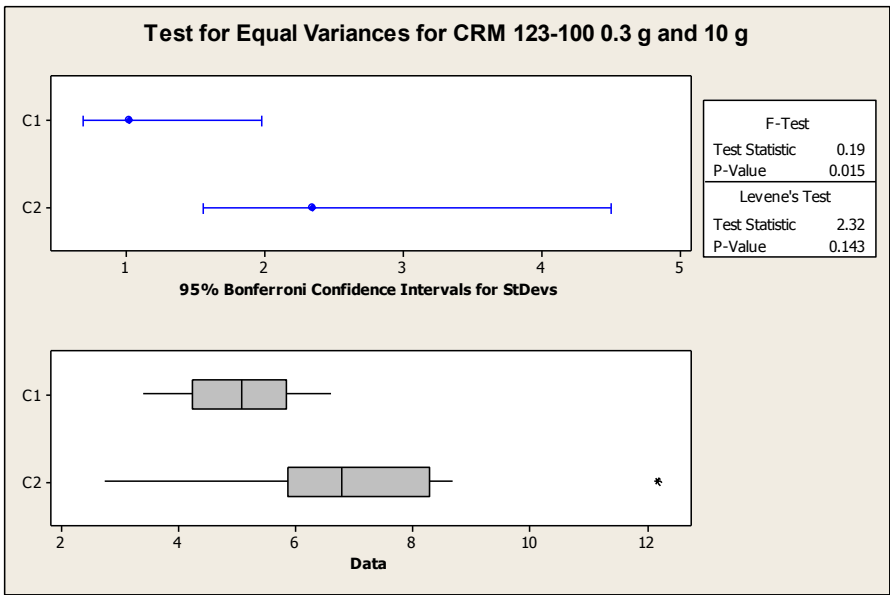


Figure 6.4: Statistical comparison of the values resulting from the extraction of PAHs by *in-situ* PFE-GC-MS of 0.3 and 10 g of Certified Reference Material (CRM 123-100)

However, in this study, a comparison was done between the total PAH content and PAH bioaccessible fractions obtained from BGS soils in two different laboratories (present study and BGS). This comparison will control the quality of the results from the present study, as results from the CRM cannot be used in this bioaccessibility study. This was part of the utilization of an interlaboratory tool to estimate the FORES(h)t method robustness.

6.3.2 Performance of analytical method following UBM

6.3.2.1 Liquid-liquid extraction

The results for the spiked reagent water and gastrointestinal fluids gave precise and accurate results with liquid-liquid extraction. All recoveries ranged from 80 to 110 % with RSD ranging from 8 to 22 % with water only, and using gastrointestinal digests similar recoveries and relative standard deviation were obtained (Figure 6.5). It has confirmed the precision and accuracy of the method for extraction of PAHs from liquid phases. Therefore, this extraction is suitable for further works with the UBM. However this technique takes a considerable amount of time because it is not automated.

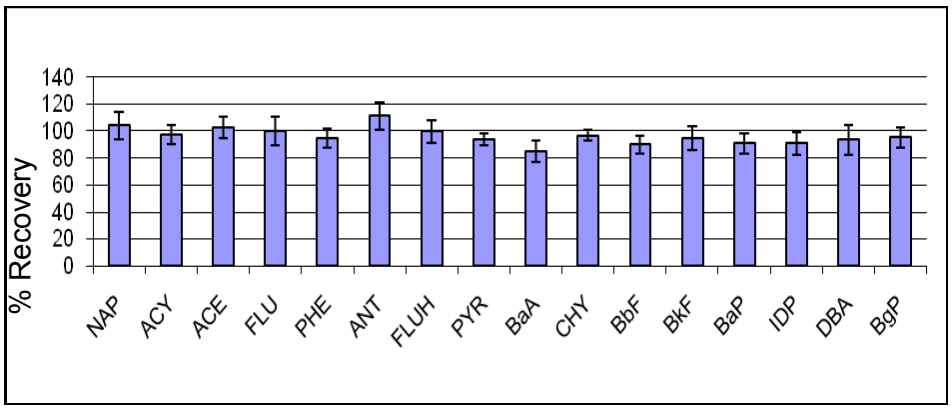


Figure 6.5: Recoveries of PAHs after Liquid-liquid extraction with (mean +/- sd, n = 3)

6.3.2.2 Solid Phase Extraction

The SPE gave also efficient recoveries ranging from 71.6 to 96.5 % and RSD ranging from 4.4 to 27.8 % (Table 6.4 and Figure 6.6) with the C18 sorbent. Indeed, the C18 end-capped octadecyl is very retentive of non-polar compounds because of its hydrophobic character. The recoveries were lower for C8 between 40 and 70 % (Figure 6.6) and again lower concerning C2 between 30 and 60 %. Due to their structure, these sorbents were expected to retain less PAH as their chain length is shorter and C2 is more polar due to the exposition of the polar group Si-O. So there is no place for hydrophobic attractions as with the C18 sorbent. C8 and C2 sorbent were therefore discarded for further analysis. However, the SPE technique with C18 sorbent was kept as a competing device against LLE. Furthermore, the advantage of SPE against LLE is the use of a vacuum manifold which can process several samples at the same time. It can reduce significantly the time compared to LLE. However, this needs to be taken with precaution, as doing the extraction on several cartridges at the same time on the vacuum manifold can involve discrepancies on the flow rate as pressure will vary according to the position of the cartridges on the manifold.

Table 6.4: Recoveries and relative standard deviation of a spiked aqueous solution (10 ml) after SPE (C18)-GC-MS

Spiking procedure SPE-GC-MS		
	Recoveries considering final concentration: 5 mg/kg	**Relative Standard Deviation**
	%REC (n=3)	%RSD (n=3)
NAP	71.6	8.6
ACY	81.2	4.4
ACE	83.5	13.4
FLU	91.5	11.0
PHE	85.8	6.9
ANT	96.5	15.1
FLUH	88.8	12.7
PYR	87.2	7.3
BaA	82.8	13.6
CHY	86.6	13.8
BbF	87.2	18.4
BkF	89.6	12.1
BaP	91.3	10.0
IDP	87.5	21.4
DBA	88.0	27.8
BgP	89.9	15.0

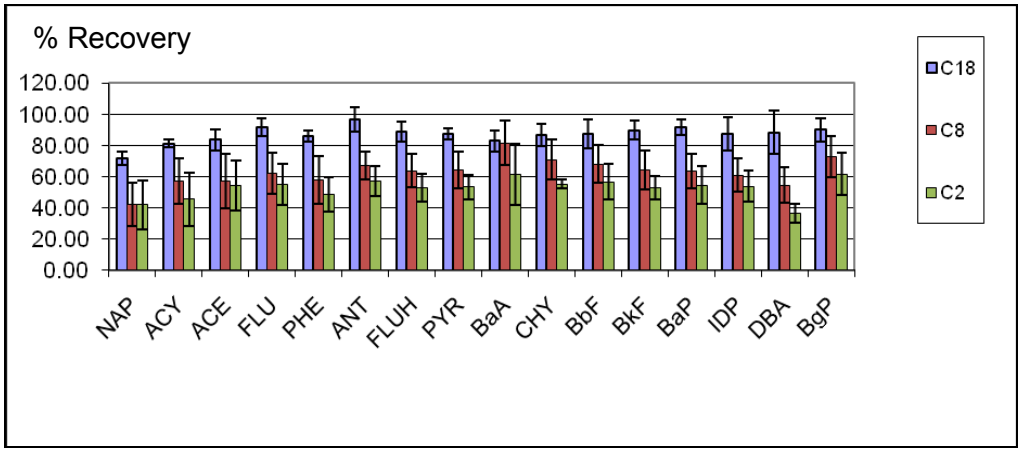


Figure 6.6: Recoveries of PAHs after Solid Phase Extraction for three types of sorbents (C18, C8 and C2) with (mean +/- sd, n = 3).

6.3.2.3 Stir-Bar Sorptive Extraction

The SBSE technique was investigated with liquid desorption in this study. Conclusions rapidly appeared that this type of desorption was not ideal. SBSE is a solvent-free method which consists of desorbing directly the stir bar without solvent into the instrument. Therefore, the use of solvent to desorb PAHs did not give very precise,

accurate and linear results, compared to the use of LLE or SPE. However, this technique could be useful in conjunction with a thermal desorption unit.

6.3.2.4 Solid Phase Micro-Extraction

Considering only the results on standards for calibration, the technique seemed more suitable assessing low quantities of contaminants in samples. In a number of studies fibre overload has been demonstrated to lead to a bias in the results (Roberts et al., 2000). The soils and CRM contained mg/kg levels of PAHs. So the needle can be easily overloaded at a certain PAH concentration and some of the PAHs will not reach the needle. Then, it could lead to incorrect results for mg/kg ranges of concentration. µg/kg concentration was giving acceptable calibration curves correlation coefficient (from R^2 = 0.95 to 0.99) avoiding overloading the needle. It was also noted that stirring time is important for the mobilization of PAHs onto the fibre. It was noticed that as stirring time was increasing (0, 5, 10, 15, 20 until 60 minutes) the amount of PAHs adsorbed onto the fibre was getting higher (value at 60 minutes up to six fold the value at 5 minutes).

6.3.2.5 Micro-extraction by Packed Sorbent

The chromatograms obtained with MEPS-PTV-GC-MS showed a very well defined baseline with sharp and well-separated peaks for all the 16 PAHs. The method allows injection of large volume of samples which increases sensitivity. The comparison of the integration of the fluoranthene peak with split/splitless mode (0.5 mg/kg) and the program temperature vaporizing/Large volume injection (0.1 mg/kg), demonstrates that the sensitivity had dramatically increased (more than 15 times using ratio of peak surface areas) and the signal-to-noise ratio was only 3 for the former method and reaching 77 for the latter (Figure 6.7). This method is therefore very useful to increase sensitivity, which is a common issue when working with samples with low concentrations of contaminants. That method can also be used in backflush mode in order to remove impurities, by venting large amount of solvent.

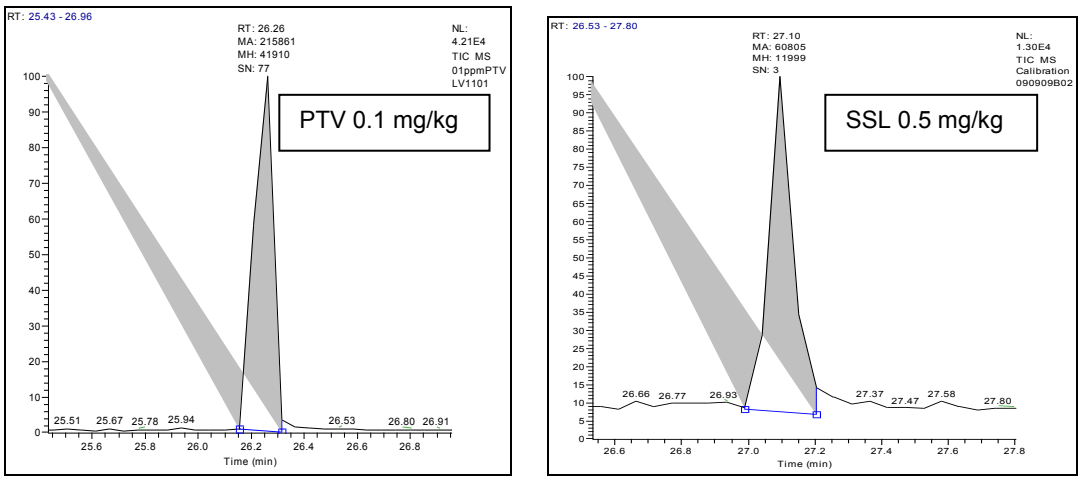


Figure 6.7: comparison of sensitivity, surface area and signal-to-noise ratio of a fluoranthene peak using split/splitless injector (SSL) (0.5 mg/kg) and Programme Temperature Vaporizing/ Large volume injector (PTV/LV) (0.1 mg/kg) with a Trace GC-Polaris Q MS for analysis.

As the technique is complex, a complete study would need to be done on this technique, before applying it on real samples.

6.3.2.6 Conclusion

As a conclusion, the best technique to isolate polycyclic aromatic hydrocarbons from gastric and intestinal aqueous solutions under these conditions was Solid Phase Extraction. Firstly, the SPE method was easy to use and did not require specific complementary devices (Stir Bar Sorptive Extraction) or use of low compounds concentration as with Solid Phase Micro Extraction. Secondly, very good recoveries were obtained with the C18 octadecyl sorbent with SPE. Finally, the method can process several samples at the same time and involves less manual operation and use of solvent, compared with liquid-liquid extraction.

6.3.3 Evaluation of bioaccessibilities using the UBM

The bioaccessible fractions for the 16 PAHs, obtained using the Unified Barge Method, were calculated using the Equation 6.1 (based on the total content in soil and the concentration obtained after using the PBET) showed very low bioaccessible fractions values from 0.73 to 7.45 % (Table 6.5). PAHs concentrations in the aqueous phase resulting from the simulated *in vitro* gastrointestinal models were also low, from

0.22 to 1.94 mg/kg. The recoveries of the addition of the residual fraction digest and the gastrointestinal digests, compared with the total PAHs content was showing values from 82.57 to 110.20 % which meant that the PAHs were remaining in the soil and were not leaching into the aqueous phase. Moreover, the % Residual was showing values between 77.03 to 110.20 %, with an exception for acenaphthene at 44.23 %, which again demonstrate that PAHs were remaining in the residue after using the physiologically-based extraction model.

$$\% \text{ BAF (Bioaccessible fraction)} = \frac{\text{Amount released using the UBM (mg/kg)}}{\text{Total content in soil (mg/kg)}} * 100 \quad [6.1]$$

Moreover, these results confirm that the use of SPE C18 sorbent to recover PAHs from the gastrointestinal digests using the Unified Barge Method is precise and accurate. As the PAH content in the gastrointestinal digests is nearly negligible after simulated extraction, this experiment can be seen as a spiking procedure, drawing two conclusions at the same time. On the one hand, SPE with C18 sorbent is definitely appropriate for the analysis of PAHs in the gastrointestinal digests resulting from the physiologically-based extraction tests. On the other hand, a fasted model involves negligible mobilizations of PAHs from soils in the gut.

As polycyclic aromatic hydrocarbons are hydrophobic compounds they are not very soluble in water, they will tend to remain in the soil matrix as they will not be attracted by a polar solvent such as water. For example, fluorene have a high solubility in water compared with other higher molecular weight PAHs, and was giving the highest bioaccessible fraction, at 7.45 %. Comparing with previous studies, based on the use of fasted *in vitro* gastrointestinal tests, researchers were finding low PAH bioaccessible fraction from 0 to 20 *%* (Hack *et al.*, 1996; Oomen *et al.*, 2004; Van de Wiele *et al.*, 2004). However, a few studies were finding higher bioaccessible fraction from up to 50 % (Gron *et al.*, 2003; Pu *et al.*, 2004; Tang *et al.*, 2006). Phenanthrene was showing particularly high bioaccessibilities compared to other polycyclic aromatic

hydrocarbons (Gron *et al.*, 2003; Pu *et al.*, 2004). It was observed that adding food in the digestive tract was increasing significantly the PAHs bioaccessibilities (Hack *et al.*, 1996; Versantvoort *et al.*, 2004).

Table 6.5: Analysis of the most contaminated Tar works soil using *in-situ* pressurized fluid extraction and the Unified Barge Method.

Most Contaminated Tar works soil						
	Total (PFE)	**Gastric + Intestinal digest (UBM)**		**Residual fraction digest (PFE)**		**%Recovery**
	Mean ± SD(n=3) (mg/kg)	**Mean ± SD(n=3) (mg/kg)**	**%BAF***	**Mean ± SD(n=3) (mg/kg)SD(3)**	**% Residual+**	**%REC^**
NAP	17.4 ± 11.8	<LOD	ND*	19.1 ± 2.2	109.8	ND
ACY	3.8 ± 1.9	<LOD	ND	3.4 ± 0.3	89.5	ND
ACE	5.2 ± 3.1	<LOD	ND	2.3 ± 1.4	44.2	ND
FLU	7.4 ±1.9	0.54 ± 0.06	7.45	5.7 ± 0.4	77.0	86.9
PHE	38.6 ± 9.9	0.79 ± 0.08	2.15	32.1 ± 2.3	83.2	88.5
ANT	35.1 ± 11.9	0.22 ± 0.07	0.69	27.0 ± 3.3	76.9	82.6
FLUH	209.3 ± 43.8	1.48 ± 0.28	0.73	181.5 ± 11.5	86.7	89.6
PYR	191.9 ± 38.3	1.94 ± 0.22	1.04	162.0 ± 10.6	84.4	87.8
BaA	106.6 ± 24.3	1.06 ± 0.10	1.02	97.9 ± 8.8	91.8	95.4
CHY	98.0 ± 14.6	1.03 ± 0.12	1.07	86.9 ± 6.8	88.7	90.5
BbF	141.6 ± 26.6	1.29 ± 0.11	0.94	131.4 ± 3.6	92.8	95.9
BkF	48.2 ± 6.7	1.21 ± 0.04	2.55	44.4 ± 3.5	92.1	96.6
BaP	184.6 ± 32.2	1.62 ± 0.22	0.90	176.2 ± 16.6	95.4	97.3
IDP	76.8 ± 12.5	1.28 ± 0.06	1.70	77.5 ± 2.6	100.1	104.2
DBA	23.42±3.76	ND	ND	25.81 ± 2.43	110.2	110.2
BgP	70.5 ± 14.1	0.57 ± 0.04	0.83	72.9 ± 5.6	103.4	106.3

* %BAF: stage related bioaccessibility, calculated as a fraction of the total (mean; n=3)

+ %Residual: residual fraction calculated as a fraction of the total (mean; n=3)

^ %Rec: calculated as a fraction of the total (mean; n=3)

* ND= non detected

6.3.4 Performance of the analytical method following FORES(h)t

The performance of the method was estimated by using a spiking procedure (Table 6.6). Recoveries were satisfactory with values from 63.0 % to 114.0 %. Only naphthalene was found at a low recovery (19.8 ± 50.4) because it was lost during evaporation due of its high volatility. Therefore, the analytical method was showing good performance as recoveries were accurate (between 70 and 130 %) and relative standard deviation were precise (< 30%), between 8.7 % and 17.1 % conform to the

USEPA criteria for the quality control and validation of analytical methods (Shoemaker, 2002).

Table 6.6: Recoveries and relative standard deviation of a spiked soil FORES(h)t Saponification-SPE (polymeric-silica)-GC-MS

Spiking procedure: FORES(h)t-Saponification-SPE-GC-MS		
	Recoveries considering final concentration: 20 mg/kg	**Relative Standard Deviation**
	%REC (n=5)	%RSD (n=5)
NAP	19.8	50.4
ACY	63.0	15.7
ACE	72.1	13.6
FLU	73.1	8.7
PHE	79.2	9.3
ANT	86.7	13.3
FLUH	94.6	11.9
PYR	92.1	10.7
BaA	99.1	13.9
CHY	109.9	15.1
BbF	114.0	13.4
BkF	107.0	15.5
BaP	97.2	13.0
IDP	111.6	14.3
DBA	95.5	16.1
BgP	97.6	17.1

6.3.5 Evaluation of PAHs bioaccessible fractions using FORES(h)t

6.3.5.1 Comparion of bioaccessible fractions with residual digests and total content

Using the FORES(h)t method, the bioaccessible fractions of PAHs inside the gastrointestinal digests, containing food, water and biological juices, were significantly higher. Concerning the 6 Tar works soils samples (1, 2, 3, 4, 5, 6) the maximum bioaccessible fractions were ranging from 9.3 % to 83.9 % (Table 6.7 (A)) and the maximum residual fraction was ranging from 43.0 % to 122.9 %. Concerning the four BGS sample soils, maximum bioaccessible fractions were ranging from 24.9 % to 103.3 % and maximum residual fractions were ranging from 41.2 % to 63.1 % (Table 6.7 (B)).

Table 6.7: Comparison of stage related bioaccessibility and residual fraction of polycyclic aromatic hydrocarbons in the St Anthony's Tar works (A) and BGS soils (B)

(A)

	St Anthony's Tar works soils									
	Total (PFE) (mg/kg) n =6			Gastric + Intestinal digest (FORES(h)t) (mg/kg) n = 6				Residual digest (PFE) (mg/kg) n = 6		
	Minimum ± SD	Median	Maximum ± SD	Minimum ± SD	Median	Maximum ± SD	%BAF*	Minimum ± SD	Median	Maximum (%residual+) ± SD
NAP	2.5±0.1	4.0	24.0±3.6	ND	3.3	4.1±1.1	9.3	ND*	4.7	18.6±0.8 (77.39)
ACY	1.5±0	3.1	5.6±0.1	ND	3.1	4.3±3.7	45.9	ND	2.3	4.3±0.3 (77.02)
ACE	2.2±1.1	3.7	7.9±0.7	ND	2.2	2.7±0.7	59.4	ND	3.0	7.7±9.3 (122.87)
FLU	3.3±0.0	5.9	14.5±1.0	3.1±0.7	3.7	5.1±0.5	65.0	ND	4.5	5.9±1.1 (96.24)
PHE	5.9±0.2	36.0	54.0±4.5	7.4±1.9	12.7	36.6±2.2	83.9	ND	21.7	27.5±3.9 (67.63)
ANT	3.9±0.1	15.2	24.6±1.2	2.8±0.7	4.2	6.2±1.4	72.1	ND	8.1	13.8±0.4 (56.13)
FLUH	9.3±0.2	56.6	242.6±7.2	3.3±0.5	10.4	55.0±6.6	35.0	3.7±0.2	35.8	104.4±2.0 (43.04)
PYR	8.0±0.1	42.6	234.1±7.4	3.2±0.7	8.1	61.1±8.8	39.9	3.1±0.1	26.8	101.7±1.5 (43.46)
BaA	5.7±0.1	31.6	102.6±6.9	2.3±0.3	7.4	65.3±2.5	63.9	4.1±0.1	20.8	50.4±1.5 (49.11)
CHY	5.9±0.1	24.1	94.8±3.2	2.2±0.1	8.0	61.3±5.6	68.7	3.5±0.1	16.2	46.1±1.5 (48.68)
BbF	5.5±0.8	27.0	117.2±3.4	2.2±0.6	6.4	67.3±1.9	57.5	4.4±0.7	17.2	58.0±0.9 (49.51)
BkF	5.1±0.6	16.8	107.7±8.5	2.3±0.2	5.7	72.5±3.1	67.5	3.5±0.1	13.8	51.5±2.0 (47.83)
BaP	5.9±0.5	32.9	141.8±12.8	1.7±0.4	6.8	69.6±8.5	49.6	3.6±0.0	20.8	80.8±2.1 (56.99)
IDP	5.2±0.5	21.1	98.0±8.9	2.1±0.3	5.4	73.3±5.3	75.4	3.0±0.4	14.6	48.8±3.2 (49.81)
DBA	4.0±0.4	9.1	26.4±2.0	1.8±0.2	3.8	6.4±1.6	76.3	1.4±0.1	7.9	16.0±0.6 (60.60)
BgP	4.2±0.1	16.9	89.0±6.7	1.6±0.4	4.3	53.1±1.2	60.0	2.6±0.1	10.5	46.0±1.9 (51.75)

*ND= non detected

Table 6.7 (continued): Comparison of stage related bioaccessibility and residual fraction of polycyclic aromatic hydrocarbons in the St Anthony's Tar works (A) and BGS soils (B)

(B)

	BGS sample soils									
	Total (PFE) (mg/kg) n =4			Gastric + Intestinal digest (FORES(h)t) (mg/kg) n = 4				Residual digest (PFE) (mg/kg) n = 4		
	Minimum ± SD	Median	Maximum ± SD	Minimum ± SD	Median	Maximum ± SD	%BAF*	Minimum ± SD	Median	Maximum (%residual*) ± SD
NAP	2.5±0.8	15.4	21.2±2.0	ND	1.6	5.4±3.3	ND	ND*	4.4	4.8±2.0 (41.2)
ACY	3.1±0.2	7.7	17.7±0.8	ND	2.5	6.0±2.1	61.8	2.0±0.2	4.0	7.7±0.5 (43.6)
ACE	2.3±0.0	4.1	4.4±0.5	ND	2.4	3.2±0.3	59.5	ND	1.6	2.7±0.2 (63.1)
FLU	3.9±0.1	6.6	7.9±0.6	ND	4.4	6.2±0.6	69.9	ND	3.0	4.5±0.2 (57.2)
PHE	22.0±1.7	25.4	27.3±3.7	8.2±0.8	13.0	22.4±3.7	103.3	2.6±0.4	7.4	13.6±0.5 (49.8)
ANT	10.4±1.0	11.4	12.1±1.1	2.4±0.2	3.0	4.2±0.4	41.0	1.8±0.4	4.9	6.4±0.4 (52.8)
FLUH	47.4±5.2	58.5	105.0±7.2	11.2±1.0	11.9	19.5±6.2	25.0	13.3±1.2	25.	38.6±1.5 (36.7)
PYR	46.8±4.8	58.8	82.4±5.8	10.4±0.6	13.0	15.7±4.1	26.0	10.1±1.2	25.0	29.5±1.0 (35.8)
BaA	28.0±0.6	31.5	53.1±3.8	6.9±0.7	7.4	11.8±3.8	27.2	8.8±0.8	15.1	23.5±1.0 (44.2)
CHY	24.9±1.2	31.9	53.3±3.3	7.4±0.9	8.8	11.0±3.1	36.6	8.2±0.5	13.5	21.0±1.2 (39.3)
BbF	32.3±5.0	48.2	51.4±2.2	8.1±0.3	12.4	13.4±2.0	26.5	11.0±1.2	18.9	29.1±2.2 (56.6)
BkF	25.7±2.9	28.0	41.5±5.5	5.9±0.4	8.8	9.8±2.7	33.7	7.8±1.3	14.6	22.6±3.5 (54.3)
BaP	38.8±5.1	59.5	62.1±5.2	6.6±0.1	12.1	15.0±2.7	24.9	8.5±1.2	25.9	27.0±1.8 (43.5)
IDP	32.4±0.5	41.0	48.6±2.2	8.5±2.3	9.4	11.2±2.7	26.3	9.2±1.1	18.6	27.0±1.9 (55.6)
DBA	8.8±0.6	9.8	10.8±0.9	1.6±1.4	2.2	2.4±0.2	26.3	2.7±0.2	5.8	6.4±0.4 (63.0)
BgP	33.8±1.1	35.6	49.8±2.3	6.5±0.7	8.7	9.8±2.3	29.0	9.0±1.4	13.7	22.7±1.1 (45.5)

*ND= non detected

Highest bioaccessible fractions were very often observed for phenanthrene in any type of soil. Bioaccessible fraction for phenanthrene were respectively for soils samples 1, 2, 3, 4, 5, 6 (Tar works soils) and 1, 2, 3, 4 (BGS soils): 64.0 %, 83.9 %, 21.9 %, >100 %, 31.0 %, 25.1 %, and 67.2 %, 103.3 %, 30.3 %, 36.2 % (Table 6.8). Unexpected high bioaccessibility for phenanthrene, compared with other PAHs, was also observed in the literature (Gron *et al.*, 2003).

Table 6.8: *In vitro* gastrointestinal extraction (FORES(h)t method): application to soil samples from St Anthony's Tar works and from BGS.

Phenanthrene				
Samples (sites)	**Total (PFE)**	**Gastric +Intestinal digest (FORES(h)t)**	**Residual digest (PFE)**	**%BAF**
	Mean (mg/kg) ± SD (n=3)	Mean (mg/kg) ± SD (n=3)	Mean (mg/kg) ± SD (n=3)	
TW1	11.8 ±5.9	7.4 ±1.9	6.3 ±0.3	64.0
TW2	43.6 ±2.2	36.6 ±2.2	23.7 ±0.3	83.9
TW3	31.3 ±3.2	9.6 ±0.3	25.4 ±2.6	21.9
TW4	5.9 ±0.2	> Total	ND*	>100%
TW5	40.7 ±8.8	11.9 ±2.1	27.5 ±3.9	31.0
TW6	54.0 ±4.5	13.5 ±2.2	19.7 ±4.5	25.1
BGS1	24.6 ±2.6	16.6 ±4.0	2.6 ±0.4	67.2
BGS2	22.0 ±1.7	22.4 ±3.7	3.5 ±0.4	103.3
BGS3	27.3 ±3.7	8.2 ±0.8	13.6 ±0.5	30.3
BGS4	26.2 ±2.4	9.4 ±0.7	11.4 ±0.5	36.2

*ND= non defined

The median of the bioaccessible fraction for all the PAH from the Tar work soil samples were respectively for the samples 1, 2, 3, 4, 5, 6: 52.43 %, 49.64 %, 21.48 %, 40.30 %, 15.62 %, 40.85 % (Table 6.9) and for the BGS soil samples the median bioaccessible fractions were respectively for the samples 1, 2, 3, 4: 26.15 %, 21.74 %, 26.00% and 22.62 % (Table 6.10). The type of soil seems to influence the bioaccessibility as the soils from a Gas Works (BGS) were giving median bioaccessible fractions between 21.74 and 26.15 % and soils from the Tar works were showing median bioaccessible fractions between 15.62 % and 52.43 % with values in a slightly higher range in this type of soils. The parameters that could influence these variations can be: the age of the contamination which could more or less bound the

compounds on the soil particles, named as weathering or sequestration (Tao *et al.*, 2010), the type, the structure of the soil and finally the organic matter. As described before in this study, the effect of organic matter on PAHs distributions was not clear. By comparing again the loss of ignition with total PAHs content, median bioaccessibilities of PAHs and gastrointestinal digest fractions, no correlations were appearing. The trends were even contradictory with the literature, showing sometimes higher bioaccessibility for high amount of organic matter (18.42 % LOI giving 21.48% median % BAF) and lower bioaccessibility for low organic matter content (9.39 % LOI giving 15.62 % median %BAF) **(Tables** 6.9 and 6.10).

Table 6.9: Comparison of the loss of ignition with the total PAH content, gastrointestinal digest fractions and median of bioaccessible fraction for the 16 PAHs in all the soil samples from the Tar works.

Soil sample site	% LOI	Total PAH content (mg/kg)	Gastrointestinal digest fraction (mg/kg)	Median BAF (%) for 16 PAHs
1=TW1	11.14	123	82.67	52.43
2	11.04	9.0	NA*	NA
3=TW2	18.42	1404	634.19	49.64
4=TW3	17.38	366	100.66	21.48
5=TW4	15.90	66.5	53.18	40.30
6	15.18	46.4	NA	NA
7	22.38	38.9	NA	NA
8	19.91	40.5	NA	NA
9=TW5	9.38	375	88.53	15.62
10=TW6	17.25	289	76.03	40.85
11	13.54	54.1	NA	NA
12	15.40	43.6	NA	NA
13	20.08	41.6	NA	NA
14	19.92	40.8	NA	NA
15	22.27	43.7	NA	NA
16	21.42	39.7	NA	NA

*NA= Non Available

The same absence of correlation was observed when doing the identical comparison with the total organic carbon content for the BGS sample soils (Table 6.10). The median bioaccessible fraction and the gastrointestinal digests were showing values in a very narrow range, from 103.8 mg/kg to 141.04 mg/kg for the gastrointestinal digests, and from 21.74 to 26.15 % for the bioaccessible fractions, with no correspondences regarding the variations in the total organic carbon content.

Table 6.10: Comparison of the total organic carbon content with the total PAH content, gastrointestinal digest fractions and median of bioaccessible fraction for the 16 PAHs in the BGS soil samples

BGS soils	TOC*	Total PAHs content (mg/kg)	Gastrointestinal digest (mg/kg)	Median BAF (%)
1	6.94	166.03	103.08	26.15
2	7.76	264.07	141.04	21.74
3	12.91	224.76	110.3	22.62
4	3.85	214.54	122.86	26.00

*Values taken from (Cave *et al.*, 2010): The analysis of the BGS soils was realized as follow (Cave *et al.*, 2010): 0.2 g of soil sample was extracted with 100 ml of 1:1 v/v acetonitrile / tetrahydrofuran at 50 °C in an ultrasonic bath for 45 mins. Extracts were filtered and 5 µl aliquots injected into an HPLC system with fluorescence detection. HPLC analysis was realized using a Hypersil PAH guard column (10 mm x 4 mm id) coupled to a Hypersil PAH analytical column (100 mm x 4.6 mm id) under isocratic conditions of 90% acetonitrile and 10% water at a flow rate of 1 ml/min. Fluorescence detection was achieved using an excitation wavelength of 296 nm and emission at 408 nm changing at 23.5 mins to excitation at 302 nm and emission at 506 nm for detection of indeno(1,2,3-cd)pyrene. BGS Samples 1, 2, 3 and 4 correspond to sample numbers 4, 7, 8 and 9, respectively (Cave *et al.*, 2010).

The bioaccessible fractions resulting from the FORES(h)t method were dramatically higher than the bioaccessible fraction from the Unified BARGE Method because of the addition of food constituents and also because of the changes in the composition of the gastrointestinal fluids. Food is known to contain a certain proportion of fat, especially vegetable oil, which can more easily attract PAHs that are known for their lipophilic properties. Few studies on a fed version of a simulated *in vitro* gastrointestinal model demonstrated the influence of food with the increase of PAHs bioaccessibilities (Hack *et al.*, 1996; Versantvoort *et al.*, 2004; Cave *et al.*, 2010).

An another reason for that increasing trend is related to the amount increase of reagents such as bile salts and mucine (Hack *et al.*, 1996). Indeed, bile salts can decrease the surface tension due to its surfactant properties, and therefore surface tension can become important into the mobilization of contaminants from soils (Oomen *et al.*, 2003; Oomen *et al.*, 2004). Moreover, bile salts can produce a favourable apolar environment inside the bile salt micelles which can retain easily hydrophobic contaminants such as PAHs (Oomen *et al.*, 2000) (cf Chapter 2).

However, these results need to be taken with caution, as the quality control values (CRM) were not within the range required (cf chapter 6.3.1), using 0.3 g. As explained before, the total values may have been underestimated, consequently the bioaccessible fraction values could have been overestimated. This could explain the

particularly high values of PAH bioaccessible fraction. But there is confidence on the fact that bioaccessibility is still elevated in this study, as our values were close enough to the quality control material, and the residual fractions were significantly low for almost all PAHs, compared to the residual fraction using the Unified Barge Method (Table 6.7 A and B)

6.3.5.2 Boxplot and PCA interpretation

The bioaccessible fractions of PAHs in this study were found in the same range as other studies considering the fed state of a physiologically-based extraction test, and showed that when adding food and increasing biological constituents amount, bioaccessible fractions can reach values higher than those observed considering a fasted state, which is really important to consider in human health risk assessment (Hack *et al.*, 1996; Versantvoort *et al.*, 2004; Cave *et al.*, 2010). By realizing the boxplots of the individual PAHs bioaccessible fraction, and individual PAHs content for the Tar works and the BGS soils it was possible to identify any correlations between those values. The boxplot of the individual PAHs bioaccessible fraction from the Tar Works soil samples (Figure 6.8) showed again phenanthrene with the largest upper quartile (up to 75 % bioaccessibility). Then, the following maximum upper quartiles of bioaccessible fractions appeared for acenaphthene, fluorene, benzo(a)anthracene, chrysene, indeno (1,2,3-cd) pyrene and dibenzo(a,h) anthracene between 60 and 80%. The lowest maximum upper quartile and means were observed for fluoranthene and pyrene between 30 and 40 %. Anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene and benzo(g,h,i)perylene were showing upper quartiles between 42 and 55 %. By comparing with the individual PAH concentration (Figure 6.8 and 6.9), it appeared that the highest bioaccessible fraction give in some cases the lowest total PAH content. The two highest upper quartile of individual PAH content were fluoranthene and pyrene and they were showing the two lowest upper quartile of bioaccessible fraction, as described before. The rest of the individual PAH content containing high molecular weight PAHs from benzo(a)anthracene to

indeno(1,2,3-cd) pyrene and benzo(g,h,i) perylene, were showing moderate to high upper quartiles of total content, and also moderate upper quartiles of bioaccessible fractions.

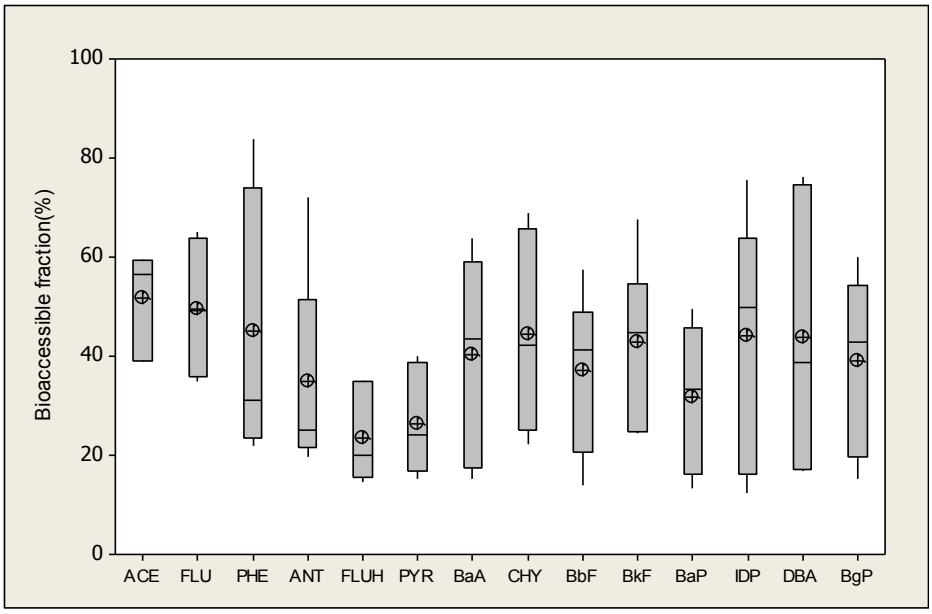


Figure 6.8: Boxplot of individual PAH bioaccessible fractions (%) in Tar work soil samples (6) with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.

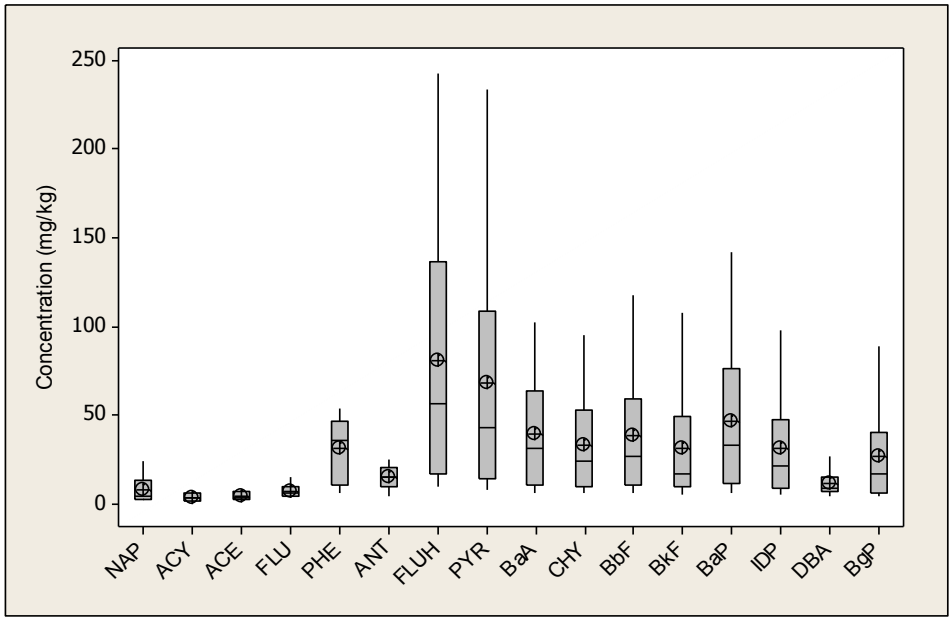


Figure 6.9: Box plot of individual PAH concentrations in Tar works soil samples (6) with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.

By using the same boxplots comparison with the BGS samples soils, a similar trend appeared more clearly. Acenaphthylene, acenaphthene, fluorene, anthracene and phenanthrene, showed the highest upper quartile of bioaccessible fractions (Figure 6.10) and the lowest upper quartiles of individual PAH content (Figure 6.11). One exception appeared for dibenzo(a,h)anthrancene which showed low upper quartile of individual content, and also low upper quartile for the bioaccessible fraction. Fluoranthene, pyrene and benzo(a)pyrene had the lowest upper quartile of bioaccessible fraction and the highest upper quartile of individual PAH content, as in the case of the Tar Works soils. The rest of the PAHs upper quartile bioaccessible fractions (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene) displayed moderate upper quartiles, and moderate upper quartiles of the individual PAH concentration.

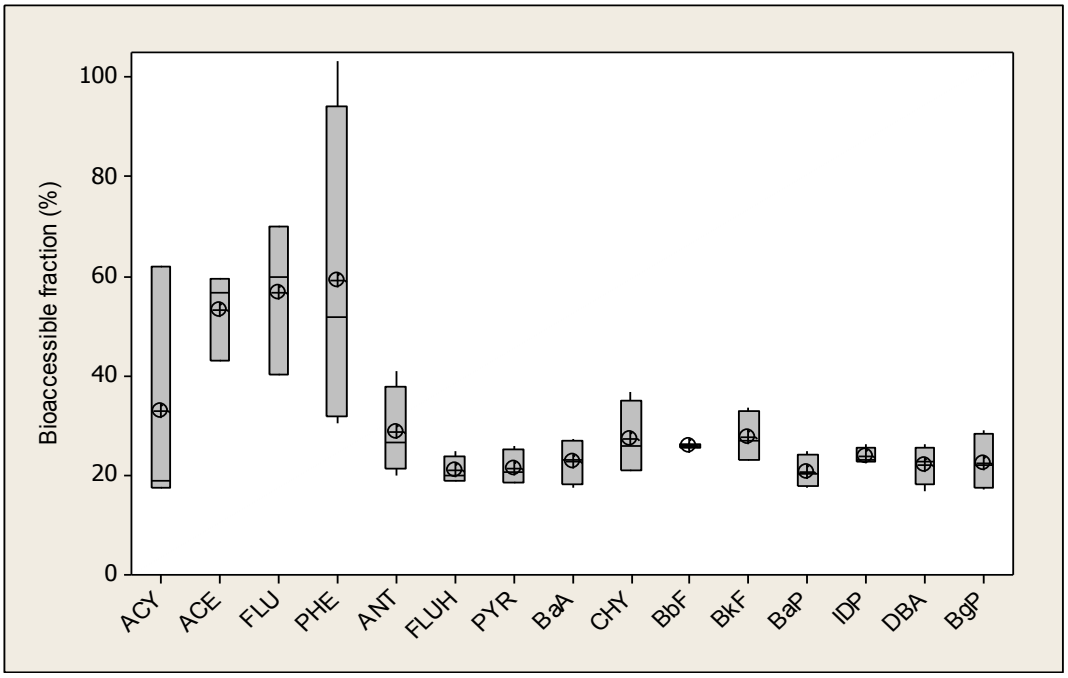


Figure 6.10: Box plot of individual PAH BAF (%) in BGS soil samples with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.

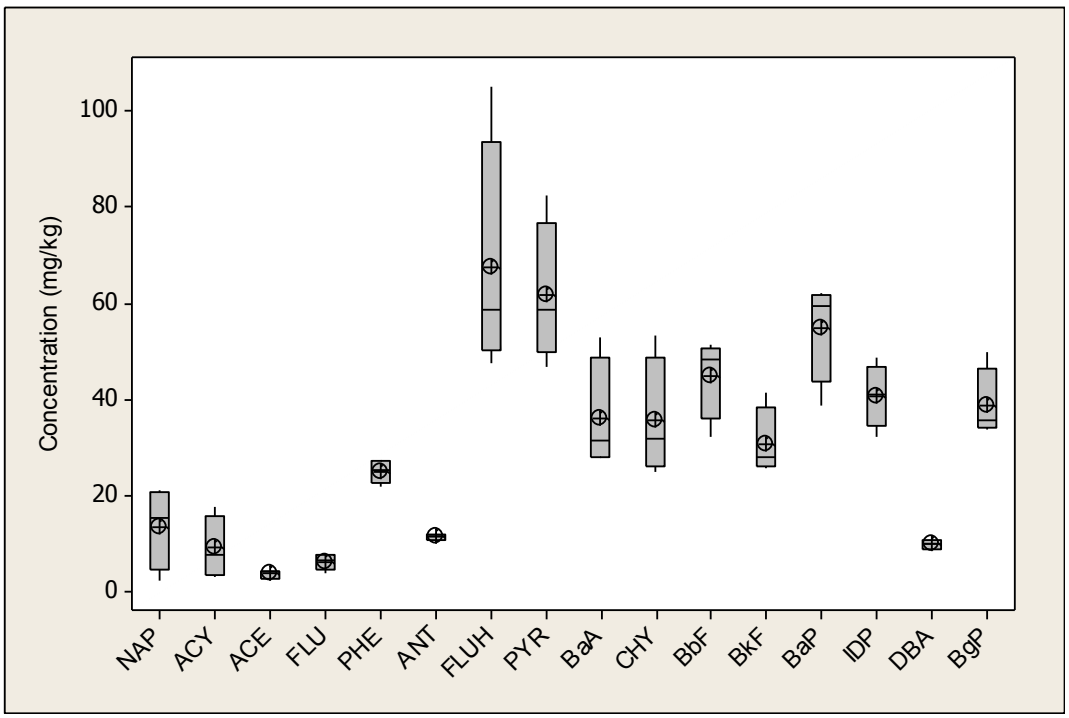
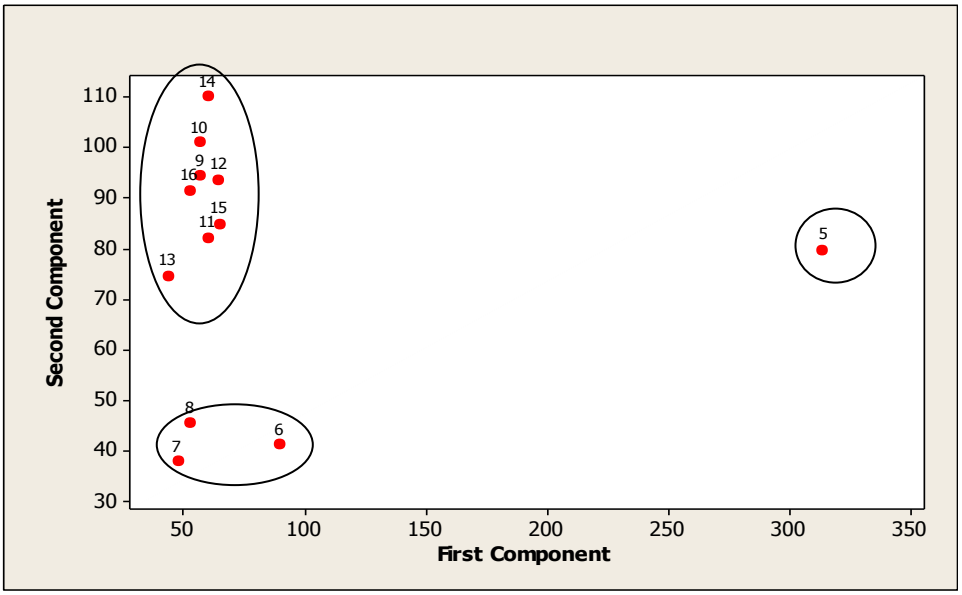


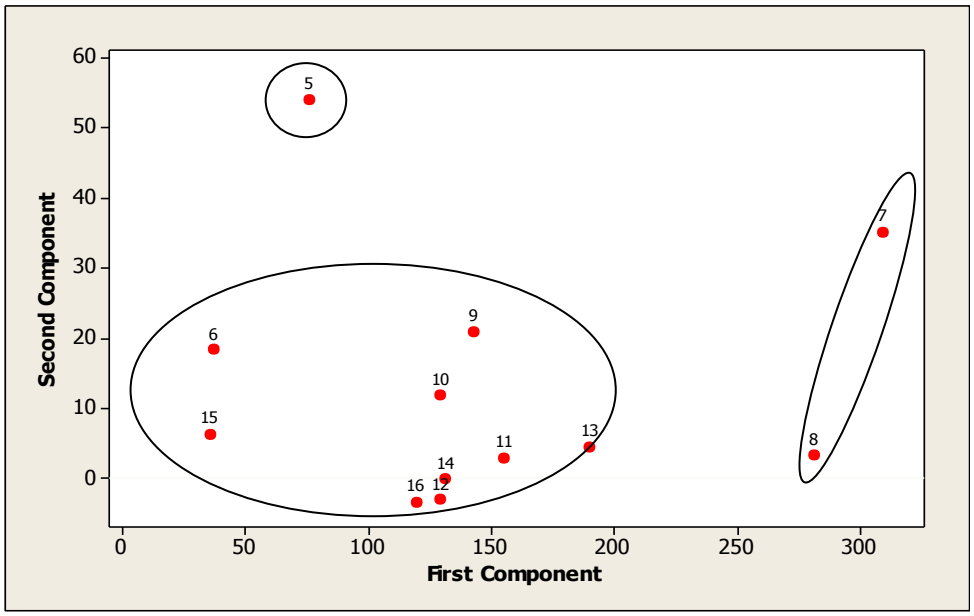
Figure 6.11: Box plot of individual PAH content in BGS soil samples with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.

A principal component analysis with covariance (Figure 6.12 and 6.13), for the individual PAH bioaccessible fraction and concentration of 14 PAHs, illustrated also the trend observed by comparing boxplots of bioaccessible fractions and individual PAH concentrations. With PCA, it appeared that three groups were formed either with the bioaccessible fractions or with the individual PAHs contents. Concerning, the bioaccessible fraction (Figure 6.12), there was one group with only phenanthrene (5), a second group with anthracene, fluoranthene and pyrene (6,7 and 8) and a third group was containing the rest of the high molecular weight PAHs.



*cf Table 6.1 for numbers corresponding to PAHs

Figure 6.12: Principal Component Analysis of each individual PAH (except the four lower molecular weights) bioaccessible fraction (%) from all soils samples (Tar Works and BGS)



*cf Table 6.1 for numbers corresponding to PAHs

Figure 6.13: Principal Component Analysis of each individual PAH content (except the four lower molecular weights) from all soils samples (Tar Works and BGS)

The PCA of the individual PAH content (Figure 6.13) unveiled also three groups: a first group only containing phenanthrene (5), a second group with fluoranthene and pyrene (6 and 7) and a last group composed by all the higher molecular weights PAH with anthracene.

Therefore, it is clear that there was a correlation between concentration in the soil matrix and the resulting bioaccessible fraction. As described previously, it seemed that a higher concentration in the soil will result in a lower bioaccessibility and conversely. This is contradictory with some studies showing increase of bioavailabilities with the increase of contaminant levels in soils (Pu *et al.*, 2004). Meanwhile, some other studies were not showing that bioaccessible fractions were independant of dose (Shu *et al.*, 1988). A possible explanation to that phenomenon implies the liquid-to-contaminant ratio parameter. As the ratio between liquid and the level of PAH will increase, it will result in higher bioaccessibility. This has been observed previously in the literature where higher bioaccessible fractions were observed for higher liquid-to-soil ratios (Van de Wiele *et al.*, 2004). Even with very low levels of contaminant in a soil, the bioaccessible fraction was still substantial (Van de Wiele *et al.*, 2004). This was linked to the dissolved organic matter present in the soils which can more or less attract contaminants such as PAHs (Van de Wiele *et al.*, 2004). Indeed, in several studies, organic matter has demonstrated an affinity or attraction of PAHs with soils (Richnow *et al.*, 1998). However, in this entire project, no correlations were found between organic matter and PAHs distributions as demonstrated previously. Other parameters that could influence the release of PAHs from the soil matrix are the solubility, the partition coefficient, the ring number and molecular weights of individual PAH (Mackay, 2001). For example, phenanthrene is very soluble in water, has a low molecular weight and ring number, compared to other high molecular weight PAHs, which would explain why its bioaccessible fraction is particularly high in many cases. This was demonstrated in a study where *in vitro* bioaccessibility of phenanthrene was close to two times the bioaccessible fraction of benzo(a)pyrene, in the digestive tract of cows (Tao *et al.*, 2010). This phenomenon was explained by the fact that the low molecular weight, lipophillicity (partition coefficient) and higher solubility of phenanthrene was increasing its bioaccessibility (Tao *et al.*, 2010). Another particular behaviour was the very high concentration of fluoranthene and pyrene as individual PAH in soils samples compared with the very

low contribution of their bioaccessible fraction. They are slightly less soluble in water than phenanthrene, and their molecular weigth and ring number is higher. This could explain why they are giving low bioaccessibilities, as they will tend to remain within the soil, as not very soluble in water and could be more strongly sorbed to the soil due to their hydrophobicity (Tao *et al.*, 2010). Indeed, these observations can be related to other PAHs properties such as the ring number and the molecular weight that could be of significant importance for the sequestration of them within soils. As described in previous chapters, predominance of pyrogenic PAHs is generally the signature of PAHs from urban and industrial areas. This is the case for the BGS soils and the soils from the Tar Works where pyrogenic PAHs are in higher concentration from fluoranthene to benzo(g,h,i)perylene (Figure 6.9 and 6.11). When observing the bioaccessible fractions it appears that the petrogenic PAHs are now in higher concentration, from naphthalene to anthracene (Figure 6.8 and 6.10). The number of rings in the structure could influence the sequestration of PAHs within the soil particles, as demonstrated in a recent study where the mobilities of high molecular weight PAHs were lower than those of low molecular weight (Tao *et al.*, 2010). This was due to higher affinities between higher molecular weight with the organic matter, and to the chemical structure of the soil that tend to retain hydrophobic compounds such as PAHs (Tao *et al.*, 2010). Indeed, higher molecular weights PAHs are more hydrophobic so they will be more sequestrated on the soil particles (Tao *et al.*, 2010). Few studies showed that bioaccessibility of PAHs were decreasing as the number of PAH ring was increasing (Tang *et al.*, 2006; Tao *et al.*, 2010).

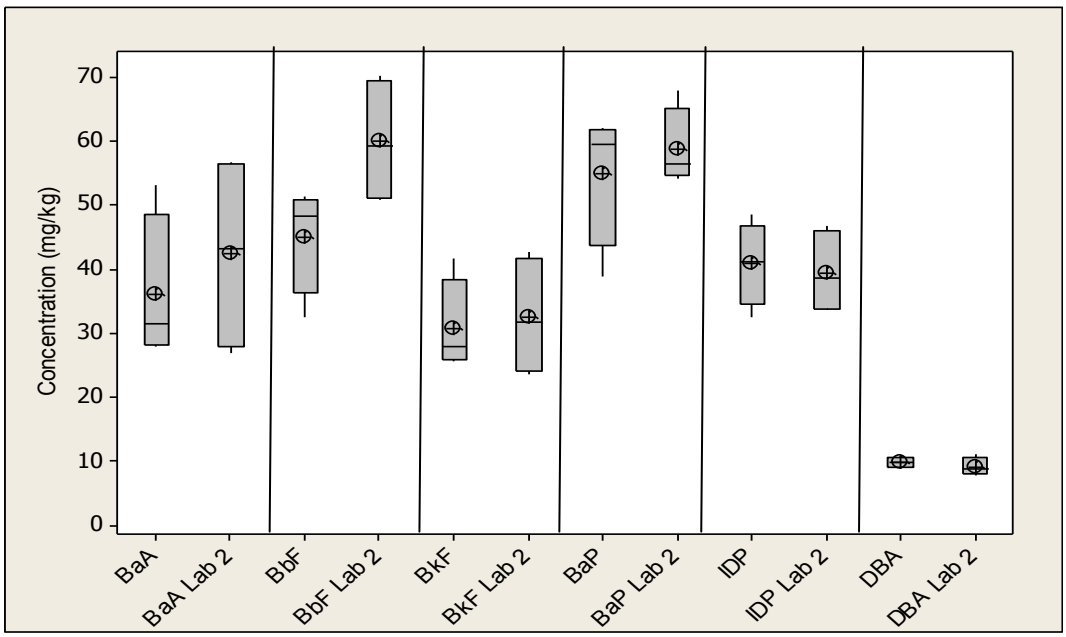
According to those comparisons, it seems complex to establish a trend on the individual PAHs bioaccessibility variations, as numerous parameters are in competitions to influence PAHs mobilization in the digestive tract. However, as a general observation, the food components seem to increase the bioaccessibility of PAHs due to the lipophilic character of the PAHs, even if some variations in solubility,

partition coefficient and ring number exist between them. Further studies would be needed to evaluate influence of each of this parameter in depth.

6.3.5.3 Interlaboratory comparison

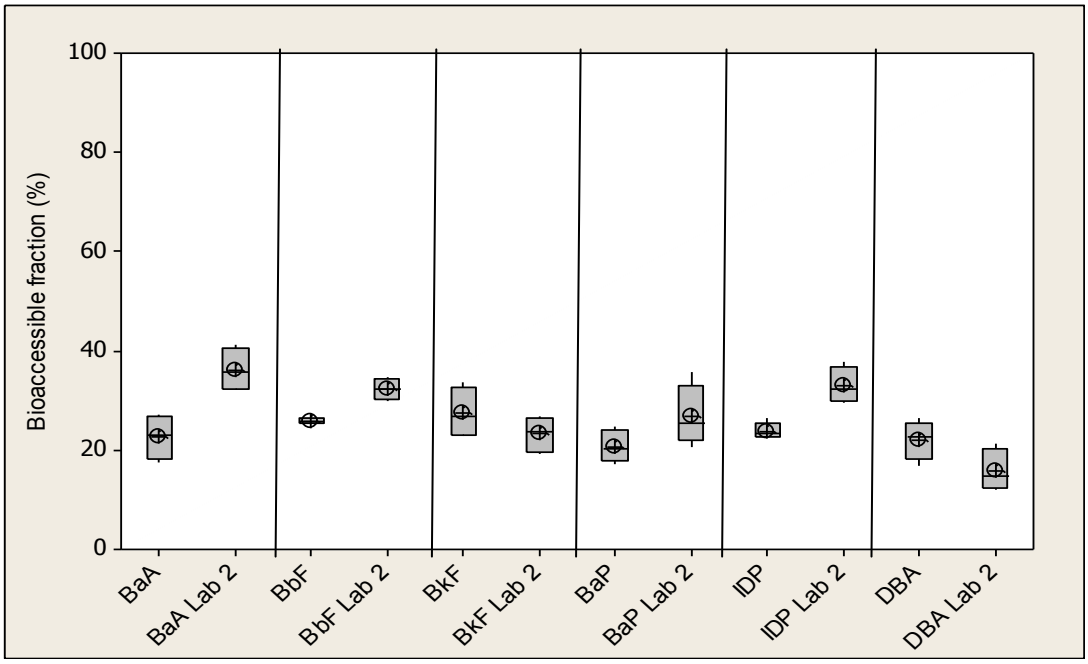
An interlaboratory evaluation was also realized for some of the PAHs compounds from the BGS soils. Bioaccessible fractions and total PAH content were compared for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene. There were two reasons to realize an interlaboratory comparison of the FORES(h)t method. On the one hand, this was done to give an indication on the performance of our laboratory and operator, using this specific method, assuring at the same time the trueness of our results. As there was no certified reference material available for PAHs bioaccessibilities for a low amount of soil (certified values based only on large quantities of CRM) this comparison will control the quality of the results obtained in Northumbria university (laboratory 1) by using the same soils than with the British Geological Survey laboratory (laboratory 2). On the other hand, this interlaboratory comparison was essential in the process of making the FORES(h)t method applicable in any commercial laboratories, by proving that the method is robust.

The individual PAH content showed similar inter-quartile range values except for benzo(b)fluoranthene where the inter-quartile range values were slightly higher in the laboratory 2 (Figure 6.14). Concerning the bioaccessible fraction, the differences were more significant, however values remained in the same inter-quartile ranges, as observed on the boxplots (Figure 6.15).



*Lab 2 values obtained using HPLC-FL (Cave *et al.*, 2010)

Figure 6.14: Boxplot of individual PAH concentration in BGS soils (Lab 2) and present laboratory with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.



*Lab 2 values obtained using HPLC-FL (Cave *et al.*, 2010)

Figure 6.15: Box plot of individual PAH bioaccessible fraction in BGS soils (Lab 2) and present laboratory with median line (50th percentile), mean cross, upper and lower quartile (25th and 75th percentile) and whiskers.

Comparison of Benzo(a)anthracene, benzo(b)fluoranthene and indeno(1,2,3-cd)pyrene inter-quartile range of values for laboratory 1 were slightly below the values for the laboratory 2. Benzo(k)fluoranthene, benzo(a)pyrene and dibenzo(a,h)anthracene inter-quartile range of values were in the same range for laboratory 1 and 2.

The slightly more significant variation in the bioaccessible fraction compared with total content can be explained by the type of method used. Indeed, to estimate the total PAH content an *in-situ* PFE-GC-MS method was used. This process did not involve as many steps as the FORES(h)t which could influence the variation of the results between laboratory 1 and 2. The FORES(h)t method involved firstly a physiologically-based extraction test which implied various steps such as shaking, heating, centrifugation and pH measurements. Then, saponification was realized on the final solution with isolation and purification of PAHs by SPE. All these manipulations can have an effect on the uncertainty of the results, therefore bringing a potential difference in results between the two laboratories. As described previously in the literature the methods of filtration and centrifugation following the simulated digestion model can introduce variability between the results from different laboratories (Cave *et al.*, 2006). However, as a preliminary study comparing the FORES(h)t method in two different laboratories, it appeared that the values were reasonably close. When observing the Figure 6.14, it showed that all inter-quartile range of PAHs bioaccessible fractions values were approximately between 18 and 41 %, and dibenzo(a)anthracene inter-quartile range of bioaccessible fraction was approximately between 12 and 26 %. It means that the bioaccessible fractions in both laboratories showed some variations but within an acceptable range, demonstrating that the method is quite robust. Further interlaboratory experiments using FORES(h)t method between laboratories would be required to validate the method.

The relative standard deviation for the recoveries of the residual fraction and the gastrointestinal digest, compared to the total PAH content, and bioaccessible fraction

(Table 6.7 (A) and (B)), were below the criteria of 30 % fixed by the USEPA (Shoemaker, 2002), so the method was repeatable in this laboratory. Moreover, the pH values were showing very good repeatability, within the required ranges, at the end of the process. Standard deviations were ranging from 0.01 to 0.02 (n=3) for the measurement at the gastric stage, they were varying from 0.02 to 0.10 (n=3) after adding bile and duodenal fluids, and finally they were situated between 0.01 and 0.05 (n=3) after shaking during two hours at 37 ± 2 °C the gastrointestinal fluids, soil and food constituents. Therefore, this work is a good start towards the elaboration of a robust fed *in vitro* gastrointestinal test that commercial laboratories could use routinely as a tool to measure human health risk from PAHs.

At the moment, comparison of *in vitro* bioaccessibilities procedures have demonstrated significant variation within and between laboratories (Environment Agency, 2005) explained partly by the variation in pre-treatment procedures applied before testing the bioaccessibility (Gron *et al.*, 2003). Only bioaccessibility testing of metals in soils (Wragg *et al.*, 2009) and pollutants in food, toys and soils (Versantvoort *et al.*, 2004) had shown satisfactory reproducibility.

6.3.5.4 Human health risk assessment

Risk assessment is the main issue when dealing with the transmission of pollutants to human via ingestion of environmental matrices. As described previously, the risk assessment is currently based either on the total concentration of pollutant in a matrix or it can be established by calculation of potential PAHs intake. Indeed, ingestion of 100 mg/day of soil has been estimated to be the average involuntary soil amount ingested per day for a young child aged between 1 and 6 years old (U.S Environmental Protection Agency, 2008). By using these values, we can calculate the amount of PAH (µg) that would be potentially ingested per day (intake), via soil, according to the individual PAH content (mg/kg) found in soil. A comparison with ingestion of 1 g and 50 g/day of soil was made, considering the case of soil-pica and geophagy behaviour (U.S Environmental Protection Agency, 2008). These calculated

values were compared with the mean daily intake of PAHs (µg) in food per day (Nathanial *et al.*, 2009). However, by using a physiologically-based extraction test, we will have access to the bioaccessible fraction and concentration, which can give more detailed informations about the human health risk, as it informs on the mobilization of PAHs in the gastrointestinal fluids and therefore on the potential maximum bioavailabilities. Indeed, by calculating directly the bioaccessible concentration in g/day, based on the weight of a small child (10 kg), the maximum amount of PAHs potentially bioavailable through the systemic circulation will be known. Those calculated values were compared with the mean daily intake of PAHs through food (µg/day), allowing a different evaluation of the potential risks of ingestion of PAHs via soils (Table 6.11 and 6.12).

Table 6.11: Amount (µg) of PAH ingested from the Tar works soils sample. Calculation are based on the maximum content of PAH (mg/kg) with assumptions of daily soil ingestion rate of 0.1 g, 1 g and 50 g (U.S Environmental Protection Agency, 2008)

	St Anthony's Tar works soils				
PAHs	**50 g/day ingestion rate***	**1g/day ingestion rate***	**0.1g/day ingestion rate***	**Bioaccessible concentration+ (g/day ingestion rate)**	**MDI (µg/day)^**
Naphthalene	1201	24	2.40	0.04	7
Acenaphthylene	281	5.6	**0.56**	0.04	0.14
Acenaphthene	393	7.9	0.79	0.03	0.98
Fluorene	727	14	**1.45**	0.05	0.59
Phenanthrene	2700	54	**5.40**	0.37	1.54
Anthracene	1231	25	**2.46**	0.06	0.08
Fluoranthene	12132	243	**24.3**	**0.55**	0.35
Pyrene	11703	234	**23.4**	**0.61**	0.35
Benzo(a)anthracene	5131	103	**10.3**	**0.65**	0.06
Chrysene	4739	95	**9.48**	**0.61**	0.11
Benzo(b)fluoranthene	5860	117	**11.7**	**0.67**	0.11
Benzo(k)fluoranthene	5386	108	**10.8**	**0.72**	0.09
Benzo(a)pyrene	7090	142	**14.2**	**0.70**	0.11
Indeno(1,2,3-cd)pyrene	4898	98	**9.80**	**0.73**	0.10
Dibenzo(a,h)anthracene	1318	26	**2.64**	**0.06**	0.04
Benzo(g,h,i)perylene	4449	89	**8.90**	**0.53**	0.06

*based on the maximum total concentration

+based on the maximum bioaccessible concentration using the gastric+intestinal digest, the calculation is based on a child weighing 10 kg.

^ Mean daily intake threshold for PAHs in food; Figures in bold represent maximum individual PAH levels that exceed the stated oral MDI

The maximum values for all individual PAH from the Tar Works, considering the ingestion of 100 mg /day of soil (U.S Environmental Protection Agency, 2008) were ranging from 0.56 µg to 24.30 µg (Table 6.10). Almost all values, except naphthalene and acenaphthene, were above the mean daily intakes of PAHs via food, therefore there would be an human health risk if those soils are ingested. Indeed, the MDI represent a limit where there will be a risk if an individual PAH concentration is above this value, and calculation of this threshold are based on the bodyweight, as for the bioaccessible concentration (Defra and Environmental Agency, 2002). The amount of PAH involuntary ingested through 100 mg/day of soil was quite high as values can reach 24.3 µg whereas the MDI only went up to a maximum of 7 µg (naphthalene), otherwise the rest of the values were situated below 1.54 µg. Therefore, when observing the Table 6.11 it appears that the risk is significant, even in the case of an involuntary ingestion of 100 mg/day of soil. The amount of PAH ingested through ingestion of 1 g or 50 g/day of soil, in the case of geophagy or soil-pica behaviour, was dramatically increased. It was obvious that, as the risk was already present for an ingestion of 100 mg/day of soil, in the case of geophagy or soil-pica behaviour the ingestion of soil will represent a serious hazard for the health of humans involved. Indeed, the amount of PAHs ingested was ranging from 5.6 to 243 µg for an ingestion of 1 g/day of soil, and from 281 to 12132 µg for an ingestion of 50 g/day of soil, which was extremely high compared to MDI values.

Considering the BGS soils, the overall values for the three different ingestion cases, were less important than in the case of the Tar works soils (Table 6.12). The maximum values of PAH ingested through soils, varied from 0.44 to 10.5 µg for 100 mg/day, 4.4 to 105 µg for 1 g/day, and 220 to 5252 µg for 50 g/day. The values still represent a risk for the three different amounts of soil ingested. Considering the involuntary ingestion of 100 mg/day of soil, again naphthalene and acenaphthene were below their respective MDI, and the rest of the individual PAH showed values above the MDI.

Table 6.12: Amount (µg) of PAH ingested from the BGS soil sample. Calculations are based on the maximum content of PAH (mg/kg) with assumptions of daily soil ingestion rate of 0.1 g, 1 g and 50 g (U.S Environmental Protection Agency, 2008)

	BGS soils				
PAHs	**50g/day ingestion rate***	**1g/day ingestion rate***	**0.1g/day ingestion rate***	**Bioaccessible concentration+ (g/day ingestion rate)**	**MDI (µg/day)^**
Naphthalene	1060	21	2.12	0.05	7
Acenaphthylene	886	18	**1.77**	0.06	0.14
Acenaphthene	220	4.4	0.44	0.03	0.98
Fluorene	393	7.9	**0.79**	0.06	0.59
Phenanthrene	1367	27	**2.73**	0.22	1.54
Anthracene	607	12	**1.21**	0.04	0.08
Fluoranthene	5252	105	**10.5**	0.19	0.35
Pyrene	4122	82	**8.24**	0.16	0.35
Benzo(a)anthracene	2656	53	**5.31**	**0.12**	0.06
Chrysene	2664	53	**5.33**	**0.11**	0.11
Benzo(b)fluoranthene	2572	51	**5.14**	**0.13**	0.11
Benzo(k)fluoranthene	2076	41	**4.15**	**0.10**	0.09
Benzo(a)pyrene	3106	62	**6.21**	**0.15**	0.11
Indeno(1,2,3-cd)pyrene	2428	49	**4.86**	**0.11**	0.10
Dibenzo(a,h)anthracene	538	11	**1.08**	0.02	0.04
Benzo(g,h,i)perylene	2490	50	**4.98**	**0.10**	0.06

*based on the maximum total concentration

+based on the maximum bioaccessible concentration using the gastric+intestinal digest, the calculation is based on a child weighing 10 kg.

^ Mean daily intake threshold for PAHs in food; Figures in bold represent maximum individual PAH levels that exceed the stated oral MDI

A more realistic approach to evaluate and refine the risk from pollutant in environmental matrices is to use the bioaccessible fraction. As we have obtained the bioaccessible fraction and concentration, using the FORES(h)t method, it is now possible to estimate the risk directly related to the potential mobilization of PAHs in the gut. The calculation of the bioaccessible concentration was based on a child weighing 10 kg. When doing this calculation, based on the maximum bioaccessible concentration of PAHs from the Tar Works (Table 6.11), values were ranging from 0.03 to 0.73 g/day. In this case, only bioaccessible concentration for fluoranthene, pyrene, chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene were above the MDIs, showing potential human health risk again for some of higher PAH molecular weigths (pyrogenic), but values were significantly lower than when

estimating the risk based on the ingestion of 100 mg/day of soil. Concerning the BGS soils the bioaccessible concentration were ranging from 0.02 to 0.22 g/day (Table 6.12), and were above MDI in some of the pyrogenic PAHs, however values were very close to the threshold. Again, a difference appeared in the estimation of the risk between bioaccessible fractions and values based on the 100 mg/day ingestion rate. These discrepancies in the risk estimation show that a consensus is needed on how to evaluate uniformly the risk from pollutants in environmental matrices, and using the most realistic and accurate approach, based on these different approaches.

6.4 Conclusion

Implementation of the Unified BARGE Method and the FORES(h)t method in the present laboratory were successful as the methods have shown efficient performance with satisfactory accuracy and precision using spiking procedures. The bioaccessible fractions have shown also good precision with RSD < 30 % for all PAHs from different locations. The interlaboratory comparison of the FORES(h)t method demonstrated acceptable reproducibility of bioaccessible fractions, for a first study in the present laboratory. Indeed, this study is going in the direction of establishing robust simulated *in vitro* gastrointestinal models that could be used routinely to estimate human health risk, as it has started to be done on other matrices and contaminants (Versantvoort *et al.*, 2004; Wragg *et al.*, 2009). Moreover, the comparison of total PAH content between the two laboratories was showing reproducible values, which can be used to further validate the methods used. This could be used as a quality tool to replace certified reference materials (if not available), in bioaccessibility testing, in further studies. Indeed, the use of a CRM with a value at 0.3 g was showing an underestimation of the real concentration on the soils, leading to a potential overestimation of bioaccessibility values, which showed again the necessity of a way to realize the quality control on bioaccessibility studies.

As a general observation, the use of a fed state of an *in vitro* gastrointestinal test has shown a dramatic increase in the bioaccessibility of polycyclic aromatic hydrocarbons from soils, compared with a fasted state. Food and biological constituents such as mucine and bile salts therefore play an important role in the mobilization of PAHs inside the digestive tract, through complex mechanisms involving absorption and adsorption, hydrophobic attractions, and sequestration. Indeed, the chemical characteristics of PAHs and soils seem to influence PAHs mobilization inside the gastrointestinal tract. However, organic matter does not show influence on the mobilization of PAHs inside the gastrointestinal fluids. For instance, the solubility in water, the partition coefficient, the molecular weight, the number of rings, and the ratio between contaminant and volume of gastrointestinal fluids could be influent parameters, providing variations in bioaccessibility, for example between higher (pyrogenic) and lower molecular weights (petrogenic). These variations demonstrated that the bioaccessibility and the total PAH can give opposite distributions, with for instance highest bioaccessibility leading to lowest PAH total content. This is important to consider, as the evaluation of the risk will lead to different conclusions, as there are multiple ways to assess the risk in a contaminated environmental matrix. Indeed, when evaluating the risk on the site using the ingestion rate (100mg/day) based on total PAH concentration, and the bioaccessible concentration, both compared to MDI values of PAHs in food, it was giving different interpretations on the risk on the site. Bioaccessible fractions estimation seems to be more appropriate and realistic to define human health risk from pollutants in environmental matrices. However, a consensus needs to be established on the estimation of the risk using bioaccessibility testing. In this study, the risk is present for both types of soils, considering the 100 mg/day ingestion rate (based on total PAHs content) with some exceptions, and in larger proportions for the Tar Works site. Using bioaccessible concentration the risk is considered lower, limited to pyrogenic PAHs for the Tar Works soils, and limited to some pyrogenic PAHs for Gas Works soils (BGS) with values very close to the MDI of PAHs in food.

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Chapter 7: Determination of PAH in urban street dust: implications for human health

7.1 Introduction

Dust is a generic term used to describe very small, solid particles (< 500 μm) which are located in the environment after deposition from airborne material. Dust attracts attention due to its potential impact on human health and can be derived from a number of sources ranging from natural, geogenic, to biogenic and anthropogenic sources. Outdoor dusts are predominantly composed of soil-derived material, as well as particles released in to the atmosphere due to volcanic eruptions and anthropogenic activity, whilst indoor dust additionally reflect personal detritus (skin flakes) as well as emissions from household appliances. Both types of dust have different compositions and involve risks to humans through direct inhalation (principally the finest particle sizes e.g. <10 μm) and unintentional consumption due to hand-to-mouth contact as well as by consuming poorly washed fruits and vegetables (< 250 μm). The focus of this chapter is on outdoor dust from an urban environment with a historic legacy of mining and industrial activity.

Outdoor dust particles can become easily airborne through wind dispersion, dispersion by road traffic as well as other activities in urban areas such as emissions from chimneys (Rogge *et al.*, 1993; Duran *et al.*, 2009; Wang *et al.*, 2009). Road side dust has been described as a complex mixture of deposited motor vehicle exhaust particles, vehicle tyre particles, spillages and leaks from vehicles including lubricating oils and fuel, road surface erosion material as well as a range of plant and animal debris and litter, including remnants of cigarette ash, all of which contain a complex range of potentially toxic elements and organic compounds including polycyclic aromatic hydrocarbons (Takada *et al.*, 1991; Rogge *et al.*, 1993; Pereira Netto *et al.*, 2006; Zhang *et al.*, 2008; Dong *et al.*, 2009; Mostafa *et al.*, 2009).

Polycyclic aromatic hydrocarbons can be classified in terms of their source as either pyrogenic or petrogenic, as described in chapter 5. The former is characterised as

being mainly derived from vehicle exhaust and combustion of fossil fuel, whereas petrogenic sources are usually derived from petroleum products and crude oil (Wang *et al.*, 2009). In terms of PAH distribution, pyrogenic sources are identified as those containing higher molecular weight PAHs i.e. those with 4 to 6 ring structures, whereas petrogenic PAHs are identified as those containing lower molecular weights PAHs i.e. those with 3 to 4 ring structures. Vehicle exhausts have been reported to be a major source of pyrogenic PAHs in street dusts from city centres (Takada *et al.*, 1991; Dong *et al.*, 2007; Hassanien *et al.*, 2008; Duran *et al.*, 2009; Mostafa *et al.*, 2009). However, a variety of other sources have also been purported to generate pyrogenic and petrogenic PAHs in road dust. Examples include tyre abrasion and tailpipe discharge (Glaser *et al.*, 2005), coal combustion products (Liu *et al.*, 2007; Zhang *et al.*, 2008), cranckage oil (Pereira Netto *et al.*, 2006; Zhang *et al.*, 2008; Mostafa *et al.*, 2009), oil combustion (Zhang *et al.*, 2008; Dong *et al.*, 2009), wood emission (Dong *et al.*, 2009), industrial emissions and the incomplete combustion of open waste burning (Hassanien *et al.*, 2008; Dong *et al.*, 2009), asphalt and tyre rubber (Dong *et al.*, 2009). Several studies have shown that the PAH distribution profile in urban road dust from both industrial and non-industrial localities shows a predominance of pyrogenic over petrogenic PAHs (Takada *et al.*, 1991; Yang *et al.*, 1995; Liu *et al.*, 2007; Zhang *et al.*, 2008; Duran *et al.*, 2009; Mostafa *et al.*, 2009; Zhao *et al.*, 2009), and the same trend is observed for other environmental matrices such as waters, soils and sediments (Yunker *et al.*, 2002; Brito *et al.*, 2005; Wang *et al.*, 2009; Lorenzi *et al.*, 2010). In contrast, some studies found mixed sources of petrogenic and pyrogenic PAHs in street dust, partly due to an inherent mixed variety of sources from both urban and industrial sites (Hassanien *et al.*, 2008; Zhang *et al.*, 2008). Typically, high concentrations of fluoranthene, pyrene and phenanthrene are markers of pyrogenic sources (Takada *et al.*, 1991; Yang *et al.*, 1995). Several studies have used ratios of selected PAHs to identify petrogenic sources as distinct from pyrogenic sources of PAHs in soils and road dusts (Blumer, 1976; Simoneit, 1985; Lipiatou *et al.*, 1991; Benner *et al.*, 1995; Budzinski *et al.*, 1997; Yunker *et al.*, 2002).

For instance, a phenanthrene / anthracene ratio of < 10 is reported to be indicative of PAHs of pyrogenic origin whereas a ratio > 15 is characteristic of PAHs of petrogenic origin (Liu *et al.*, 2007).

Traffic has clearly been demonstrated as a potential pyrogenic source of PAHs in road dust through vehicle exhausts (Pereira Netto *et al.*, 2006). However, exhaust emissions may vary according to the type of road surface, traffic volume and vehicle speed (Mi *et al.*, 2001; Dong *et al.*, 2009). Studies have linked heavily trafficked road zones with high PAH concentration (Takada *et al.*, 1991; Pereira Netto *et al.*, 2006). In a study using various engine types it was reported that increasing the speed of a vehicle can influence the dispersion of PAH emissions in the atmosphere (Mi *et al.*, 2001). However, the authors propose some caution in interpretation of the data. Also, it has recently been shown that other potential factors can affect PAH concentration in road dust, such as, the number of traffic lanes, and the street cleaning frequency (Dong *et al.*, 2009). In contrast however, high concentrations of PAH have also been found in areas without significant vehicular traffic demonstrating that other sources can influence the presence of PAHs in road dust (Dong *et al.*, 2009).

In terms of human health risk assessment, there are multiple pathways of human exposure to road dust such as inhalation, ingestion and dermal exposure. Similarly with the pollutant mobilization in soil, dust particle size is a crucial factor in the exposure pathway to humans (Driver *et al.*, 1989; Finley *et al.*, 1994; Kissel *et al.*, 1996; Choate *et al.*, 2006; Yamamoto *et al.*, 2006). Indeed, dust particle size fractions below 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$) can enter the respiratory system by inhalation (Miguel *et al.*, 1999; Plumlee *et al.*, 2006; Riddle *et al.*, 2007) whereas dust particle sizes below 250 μm (Bornschein *et al.*, 1987) adhere easily to the skin and therefore can easily be ingested through hand-to-mouth behaviour. The ingestion of environmental matrices of < 250 μm particle size is of particular interest, for example, in studies involving *in vitro* gastrointestinal extraction to evaluate potential pollutant bioaccessibilities and human health risk. An investigation of different particle sizes of

street dust, with respect to its PAH content, is consequently of importance as part of a quantitative evaluation of human health risk.

The chapter will therefore focus on (1) the analysis of urban dust from the city centre of Newcastle-upon-Tyne, (2) an evaluation of pyrogenic and petrogenic PAH distribution in this urban dust, (3) an evaluation of PAH distribution with respect to particle size and finally (4) a comparison between the PAH content of these urban dust samples with other urban environments around the world.

7.2 Experimental

List of chemicals, instrumentation and GC-MS (Trace GC; Polaris Q) analysis, have already been described in chapter 4, so they are not represented in this chapter.

7.2.1 Collection and preparation of dust samples

The dust samples were collected in the city centre of Newcastle upon Tyne, North East England (Figure 7.1) using a brush and a pan. Details about the locations of the sampling sites such as description of the location, driving speed, receptors and number of vehicle/day are explained in Table 7.1. The dust samples were air dried in a fume cupboard for one week and then sieved from < 2 mm to < 63 μm and stored in Kraft® paper bags, prior to analysis.

7.2.2 Chemicals

Two certified reference materials (CRM) (LGCQC3008 sandy soil and CRM 123-100 BNA's in soil) were obtained from LGC Standards, Teddington, UK

7.2.3 Procedure

All dust samples were analysed for the 16 priority PAHs outlined in Table 7.2. The total PAH content of dust samples was determined in the > 250 μm particle size fraction for all sample sites. Then, total PAH content was determined in a large range of particle size for sample 10, 11 and 12 for particle sizes < 63 um to < 2mm.

Table 7.1: Road dust sample locations, descriptions and possible receptors on site (Okorie, 2010).

Site number	Location	No of vehicles/ day	Driving Style	Description/sources	Possible receptors
1	Robinson Library, Claremont Road	14,091	Fast moving traffic	North of City Centre air quality Management area (AQMA). Entrance to Claremont bridge (over the Great North Road B1318) directly opposite entrance to Robinson library. Ivy (*Hedera sp*.) forming a semi protected area in which soil/dust can accumulate. Matrix comprising soil from adjacent landscaped area and curb-side dust.	Busy pedestrian thorough fair with cyclist
2	Brandling Park, Forsyth Road	28,885	Fast moving traffic	North of City Centre AQMA. Park adjacent (to the east) of the Great North Road B1318. Sample taken from a rectangular seating area. Matrix comprising soil from adjacent landscaped area and general urban inputs; sloppy sediment overlain by leaf litter.	Urban parkland. Receptors include dog walkers, pedestrians and those using the site as a general recreation area
3	Grainger Street opposite St John's Church	3,338	Restricted traffic	Sample collect from corners either side of 3 doorways and recessed areas between two buildings. Matrix: street dust and 'rubbish' (cigarette ends, litter & other detritus of plant and animal origin).	Busy pedestrian route to and from station. Busy with vehicular traffic.
4	Bolbec Hall, Westgate Road	8,629	Restricted traffic	Sample taken along base of lowest stone step. Matrix: street dust, sediment and 'rubbish' (cigarette ends, litter & other detritus of plant and animal origin).	Busy with vehicular traffic. Critical receptors pedestrians.
5	St Nicholas Church, St Nicolas Street	9,873	Restricted traffic	Sample taken along edge of building adjacent to Nicholas Road and either side of main doorway on St Nicholas Place.	Adjacent to busy pedestrian route. Busy with vehicular traffic.

Table 7.1 (continued): Road dust sample locations, descriptions and possible receptors on site (Okorie, 2010).

Site number	Location	No of vehicles / day	Driving Style	Description/sources	Possible receptors
6	Blacket Street	1,075	Restricted traffic	Sample taken in 'tunnel' (Eldon square shops above) from block paved area either side of the main road. Road access restricted to buses and taxis.	Adjacent less than 2 m away to busy pedestrian route.
7	Westgate Road opposite County Court	7,752	Restricted traffic	Samples collected from the edge of the busy road opposite to a pub. Matrix: Dust particles blown to the edges of the road	Adjacent to busy pedestrian route. Busy with vehicular traffic.
8	St James's Park, Strawberry Street	5,063	Restricted traffic	Sample taken along the edges of the road opposite St James Park and busy with vehicular traffic.	Adjacent to busy pedestrian route, especially during football match.
9	Percy Street, opposite Haymarket bus station.	1,815	Restricted traffic	This sample was collected from corners of the building directly opposite the Hay market bus station.	Receptors here are the pedestrian and those queuing for
10	All Saints Cemetery	NA*	Fast moving traffic	A few meters west of Jesmond air quality management area (AQMA). Cemetery situated along the busy Jesmond Road. Matrix: Sample of soil/sediment taken from the base of the Cemetery wall immediately to the east of the Cemetery gate.	Busy pavement walkway and cemetery entrance.
11	St Nicolas Square	NA	Restricted traffic	Sample taken in open paved square where sediment had accumulated in the uneven paving slabs. Matrix comprising soil from adjacent landscaped area and street dust	Adjacent to busy pedestrian route. Busy with vehicular traffic.
12	Central Station	NA	Restricted traffic	Sample taken under archway entrance to station adjacent to the taxi rank. Matrix: accumulated street dust	Adjacent to busy pedestrian route. Busy with vehicular traffic

*Non available

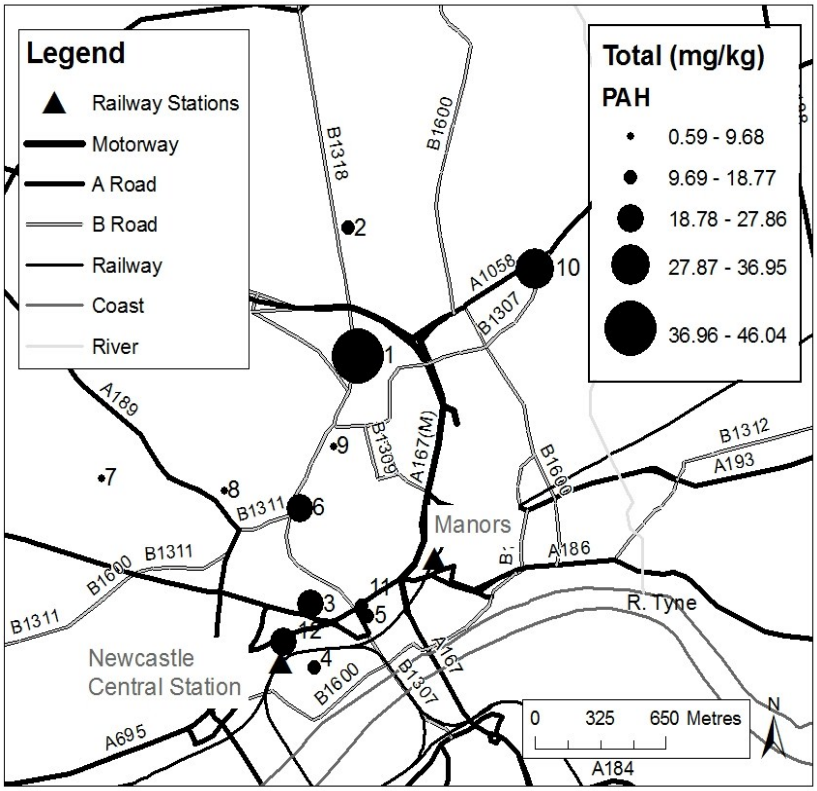


Figure 7.1: Location of the twelve dust sampling sites in Newcaslte upon Tyne, N.E. England.

Each PAHs were extracted by *in-situ* PFE followed by Gas Chromatography Mass Spectrometry (GC-MS), as described in chapter 4 and 5. Florisil (2 g) was added on top of alumina (2 g) in to the extraction cell on top of the filter paper. Then, the dust sample (2 g) was mixed with a similar quantity of high purity diatomaceous earth (Hydromatrix) and added in to the extraction cell on top of the alumina. Additional Hydromatrix was added to fill the capacity of the extraction cell and a final filter paper was placed on top prior to cell closure. PFE was performed under the same conditions that were developed for the soil matrix in Chapter 4. After PFE, the solvent (dichloromethane : acetone, 1:1, v/v) was evaporated under a gentle stream of nitrogen gas to either less than 1 ml or dryness, and then reconstituted to either 1 mL or 100 µL of DCM, according to PAH signal response, prior to the injection of 1 µL into the GC-MS.

The GC-MS was operated in selected ion monitoring (SIM) mode using the ions shown in Table 7.2 for each individual PAH. All dust sample data were reported as PAH concentration (mg/kg, dry weight). As part of the in-house quality control procedure, two CRMs were selected with a PAH of appropriate certified concentration. In accordance with the certification of the CRMs the recommended soil weight of 10 g was extracted using *in-situ* PFE with 2 g alumina.

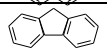
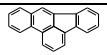
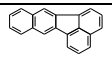
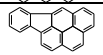
7.2.4 Organic matter content

The organic matter content of the road dust samples was determined using the same procedure described in Chapter 5.

7.3 Results and Discussion

Calibration for the determination of the 16 PAHs in a standard solution was determined. The results showed good linearity (Table 7.2) over a concentration range from 0.5 to 5 mg/kg (with 5 data points). In addition, an assessment of the sensitivity of the analytical methodology was determined in order to establish a practical lower limit of determination. In this study, the sensitivity of the GC-MS was an important parameter to consider in the determination of individual PAH concentrations. It was experimentally determined that the Limit Of Detection (LOD based on a signal-to-noise ratio equal to 3 using peak areas; calculated using Xcalibur^{TM} 1.4 SR1 software) of the instrument varied between 0.1 and 2.5 mg/kg, depending upon the individual PAH. Increased sensitivity was achievable by pre-concentration of the sample, using evaporation, to values that ranged from 0.01 and 0.17 mg/kg, depending upon the individual PAH (Table 7.2). Initial experiments focused on the development of the analytical methodology. This was done by spiking a dust sample (2 g) with 5 µL of a PAH standard solution (2000 mg/kg). The recoveries were all between 75 and 110%, except naphthalene which had a recovery of 59.0% (Table 7.3). The poorer recovery for naphthalene is due to its loss during gentle solvent evaporation post-PFE, due to its high volatility.

Table 7.2: Calibration data for analysis of PAHs by GC-MS: based on a five point graph (0.5 - 5 µg/mL).

PAH Structure	Empirical Formulae	PAHs	MS Ion for Quantitation	LOD in dust (mg/kg) (S/N > 3)*	LOD in dust (mg/kg) after evaporation (S/N > 3)*	Calibration y = mx + c	Linear regression coefficient R^2
	C10H8	Naphthalene (NAP)	128	0.7	0.04	1.3313 X + 0.0865	0.9972
	C12H8	Acenaphthylene (ACY)	152	0.2	0.02	1.3079 X + 0.0981	0.9982
	C12H10	Acenaphthene (ACE)	154	0.4	0.02	0.8795 X + 0.0880	0.9988
	C13H10	Fluorene (FLU)	166	0.1	0.01	0.9513 X + 0.1655	0.9968
	C14H10	Phenanthrene (PHE)	178	0.4	0.03	1.3456 X + 0.1703	0.9998
	C14H10	Anthracene (ANT)	178	0.2	0.01	1.0494 X + 0.1035	0.9980
	C16H10	Fluoranthene (FLUH)	202	0.1	0.01	1.1869 X + 0.1665	0.9986
	C16H10	Pyrene (PYR)	202	0.5	0.03	1.2741 X + 0.1632	0.9975
	C18H12	Benzo(a)anthracene (BaA)	228	1.7	0.11	0.7502 X + 0.1146	0.9951
	C18H12	Chrysene (CHY)	228	2.5	0.17	0.9428 X + 0.1368	0.9990
	C20H12	Benzo(b)fluoranthene (BbF)	252	0.5	0.03	0.7314 X + 0.1042	0.9949
	C20H12	Benzo(k)fluoranthene (BkF)	252	1.9	0.12	0.9363 X + 0.1443	0.9949
	C20H12	Benzo(a)pyrene (BaP)	252	0.2	0.01	0.6183 X + 0.0613	0.9971
	C22H12	Indeno(1,2,3-cd)pyrene (IDP)	276	0.2	0.01	0.5309 X + 0.0790	0.9980
	C22H14	Dibenzo(a,h)anthracene (DBA)	278	1.2	0.08	0.4932 X + 0.0782	0.9977
	C22H12	Benzo(g,h,i)perylene (BgP)	276	0.2	0.01	0.6554 X + 0.0583	0.9968

*LOD based on observations of signal-to-noise ratios of peak areas equal to 3, using the Xcalibur ™ 1.4 SR1 software.

Table 7.3: Determination of PAHs using *in situ*-PFE-GC-MS: (a) PAH recoveries from a spiked dust sample and (b) two certified reference materials (CRM LGC QC 3008 and CRM 123-100)

	Spiked dust sample		CRM LGC QC 3008 (sandy soil 2)		CRM 123-100 (BNA's in soil)			
PAH	**Recovery (%)**	**RSD (%)**	**Measured (+/- SD) n = 3 (mg/kg)**	**Certificate Value (+/- SD) n = 3 (mg/kg)**	**Measured (± SD) n = 3 (mg/kg)**	**Certificate value (mg/kg)**	**Confidence Interval (mg/kg)**	**Prediction Interval (mg/kg)**
Naphthalene	59.0	3.3	3.4 ± 0.1	3.1 ± 0.9	6.4 ± 0.8	9.73	8.49 -11.0	4.84 - 14.6
Acenaphthylene	92.5	11.1	3.9 ± 0.5	3.4 ± 1.6	2.9 ± 0.3	7.24	5.75 - 8.73	1.37 - 13.1
Acenaphthene	87.4	7.1	1.5 ± 0.3	< 2	5.0 ± 0.6	7.52	6.20 - 8.84	2.31 - 12.7
Fluorene	91.4	8.1	6.7 ± 0.4	7.7 ± 1.7	4.2 ± 0.3	6.88	5.91 - 7.85	3.05 - 10.7
Phenanthrene	96.4	4.8	28.7 ± 3.8	34 ± 7.1	4.9 ± 0.4	7.94	6.96 - 8.92	4.07 - 11.8
Anthracene	87.2	0.1	8.0 ± 0.8	5.9 ± 2.1	3.9 ± 0.4	6.94	5.90 - 7.98	2.83 - 11.1
Fluoranthene	76.1	3.2	29.2 ± 6.0	32 ± 6.4	6.2 ± 0.6	9.31	8.08 - 10.5	4.44 - 14.2
Pyrene	75.4	1.9	20.6 ± 3.5	24 ± 6.5	4.1 ± 0.3	6.75	5.79 - 7.71	2.98 - 10.5
Benzo(a)anthracene	89.4	3.9	10.2 ± 1.8	11 ± 2.5	5.1 ± 0.2	8.38	7.24 - 9.52	3.87 - 12.9
Chrysene	75.6	4.7	9.1 ± 1.1	9.9 ± 2.1	7.6 ± 0.5	11.3	10.0 -12.6	6.23 - 16.4
Benzo(b)fluoranthene	88.5	0.4	10.4 ± 1.8	9 ± 3.3	NA*	NA	NA	NA
Benzo(k)fluoranthene	100.3	15.0	6.1 ± 1.3	5.8 ± 2.2	NA	NA	NA	NA
Benzo(a)pyrene	105.7	4.2	8.3 ± 1.5	8.2 ± 1.8	4.6 ± 0.4	7.77	6.79 - 8.75	3.92 - 11.6
Indeno(1,2,3-cd)pyrene	110.0	12.7	6.6 ± 1.4	5.2 ± 1.8	NA	NA	NA	NA
Dibenzo(a,h)anthracene	97.0	22.3	3.7 ± 0.2	< 2	NA	NA	NA	NA
Benzo(g,h,i)perylene	96.3	12.5	6.1 ± 1.1	5.2 ± 1.8	NA	NA	NA	NA

*NA= non available

The precision is generally good for most PAHs with typical recoveries, based on three determinations, of < 10 %RSD, the exception is dibenzo(a,h)anthracene with an RSD of 23% (Table 7.3). In terms of measured versus certified values for the two CRMs it is noted that all PAHs are within the specified mean ± sd for CRM LGC QC 3008 whereas for CRM 123-100 all measured data were within the prediction interval for PAH content.

7.3.1 PAH content in dust samples

The concentrations of total PAHs in all twelve dust sample sites from the study area are shown in Figure 7.2. It is possible, from this data, to identify three main groups of PAH concentration. Firstly, a group having low PAH concentrations ranging from 0.59 to 2.30 mg/kg (samples 7, 8, 9); a second group having moderate PAH concentrations ranging from 15.6 to 22.5 mg/kg (samples 2, 3, 4, 5, 6, 11 and 12) and a final group having the highest concentrations which range from 36.1 to 46.0 mg/kg (samples 1 and 10). Analysis of the same data set by principal component analysis confirmed these three groups (Figure 7.3).

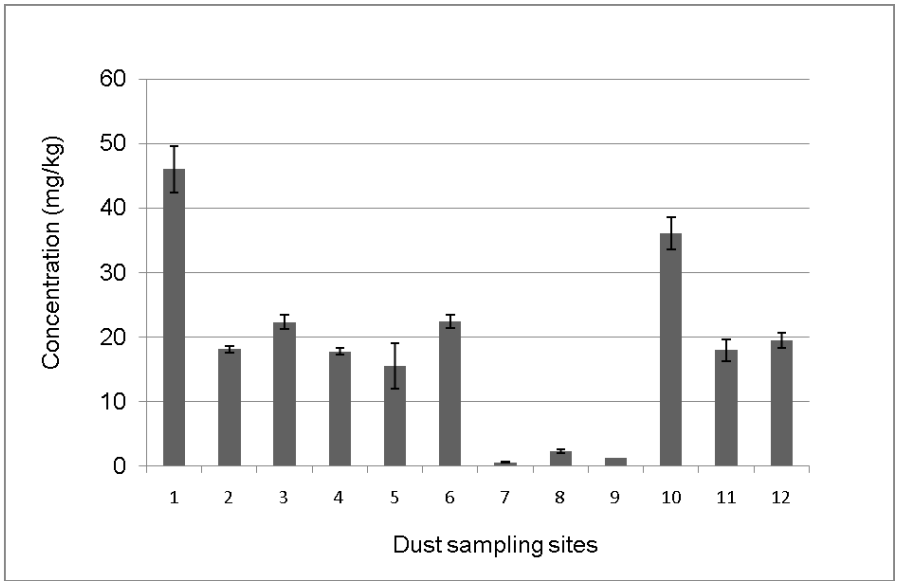


Figure 7.2: Total PAH content of the twelve dust samples (particle size > 250 µm)

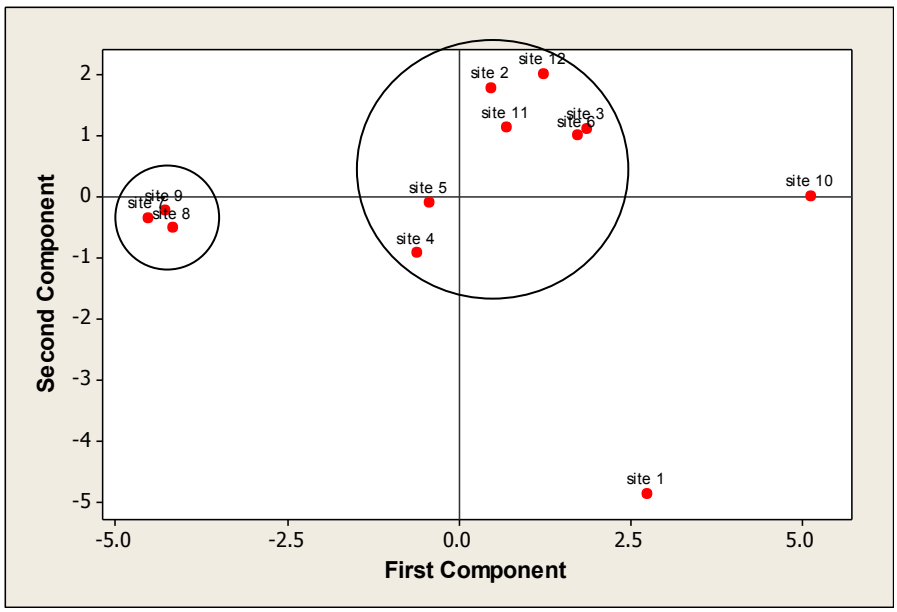


Figure 7.3: Principal Component Analysis of total PAH content in twelve dust sample

It is possible to link these results with their collection sites (Figure 7.1 and Table 7.1). The sampling sites in which the lowest PAH concentrations were determined (sites 7, 8 and 9) are all characterised as being city centre based and within close proximity to pedestrian walkways and areas of restricted traffic i.e. buses and taxis only (by inference vehicles in these areas travel at low speed < 30 km/h). The moderate PAH concentration sites (sampling sites 2-6, 11 and 12) are generally characterised as city-centred based in areas of restricted and often slow moving, traffic. The exception is sampling site 2 which was collected adjacent to a public park on a minor B-road. In contrast, sampling sites 1 and 10 (with the highest PAH concentrations) are both located on busy roads with fast moving traffic (<90 km/h). Sampling site 1 is adjacent to the A167 (M) motorway while sampling site 10 was at the junction of 3 major road tributaries which are used as major access points to the east of the city centre. A summary of the level of total PAHs in street dust from 22 locations around the world is shown in Table 7.4. The levels of PAHs in Newcastle-upon-Tyne dust samples are comparable with other cities around the world.

Table 7.4: Global determination of PAHs in roadside dust.

City, Country	Year	ΣPAHs (mg/kg dry weight)	Number of PAHs analysed	Source	Reference
Niteroi city, Brazil	2006	0.43 to 1.25	21	Urban	(Pereira Netto *et al.*, 2006)
Tokyo, Japan	1991	1.4 to 26	34	Urban, residential	(Takada *et al.*, 1991)
Dalian, China	2009	1.9 to 17	25	Urban, residential, industrial	(Wang *et al.*, 2009)
Beijing, China	2009	0.3 to 1.3	16	Urban	(Wang *et al.*, 2009)
Bangkok, Thailand	2007	1.1	10	Urban	(Boonyatumanond *et al.*, 2007)
Ulsan, Korea	2009	46 to 112	16	Industrial, residential, urban	(Dong *et al.*, 2009)
Yangtze river delta, China	2009	1.6 to 9	16	Industrial, residential, urban	(Zhao *et al.*, 2009)
Kaohsiung, Taiwan	1997	122 to 298	16	Industrial, urban, seashore	(Yang *et al.*, 1997)
Shanghai, China	2007	6.9 to 33	16	Urban	(Liu *et al.*, 2007)
Greater Cairo, Egypt	2008	0.05 to 2.6	12	Urban and residential	(Hassanien *et al.*, 2008)
8 cities, Egypt	2009	0.03 to 0.38	30	Residential and urban	(Mostafa *et al.*, 2009)
Okayama city, Japan	2008	46	4	Urban, residential	(Kose *et al.*, 2008)
Taichung, Taiwan	2004	16 to 66	21	Urban, industrial and suburban	(Fang *et al.*, 2004)
Pasadena, USA	1993	59	39	Urban	(Rogge *et al.*, 1993)
Birmingham, UK	1995	13 to 94	16	Urban	(Smith *et al.*, 1995)
Kuala Lumpur, Malaysia	2002	0.05 to 0.2	17	Urban , rural	(Omar *et al.*, 2002)
Lahore, Pakistan	1995	0.12 to 1.0	16	Industrial, urban, rural	(Smith *et al.*, 1995)
Maracay, Venezuela	2009	9.9 to 696	4	Urban	(Duran *et al.*, 2009)
Ulsan, Korea	2007	0.04 to 0.31	16	Residential and urban	(Dong *et al.*, 2007)
Santa Monica, California	2005	0.2 to 4.8	15	Residential and urban	(Lau *et al.*, 2005)
Various cities, Germany	1995	3.1 to 216	19	Urban, residential, industrial	(Yang *et al.*, 1995)
Newcastle-upon-Tyne, England	2010	0.5 to 95	16	Urban	Present study

7.3.2 Identification of PAH sources (pyrogenic / petrogenic) in road dust

The concentration of each individual PAH in the urban dust is shown in Figure 7.4. It is noted that the concentration of the low molecular weight PAHs i.e. naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene with 2 - 3 ring structures are very low (generally well below 2 mg/kg, except phenanthrene). The moderate molecular weight PAHs i.e. fluoranthene, pyrene, benzo(a)anthracene and chrysene, with 4-5 ring structures plus the 5 ring structure PAHs of benzo(b)fluoranthene and benzo(k)fluoranthene have the highest individual PAH concentrations with values up to 8 mg/kg. In accordance with the low molecular weight PAHs, the highest molecular weight compounds i.e. benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene, with 5 - 6 ring structures have concentrations well below 2 mg/kg. This trend demonstrates a predominance of pyrogenic PAHs, which are known to be produced through anthropogenic sources such as combustion of fossil fuels and vehicle exhausts (as compared to petrogenic PAHs) (Yunker *et al.*, 2002)

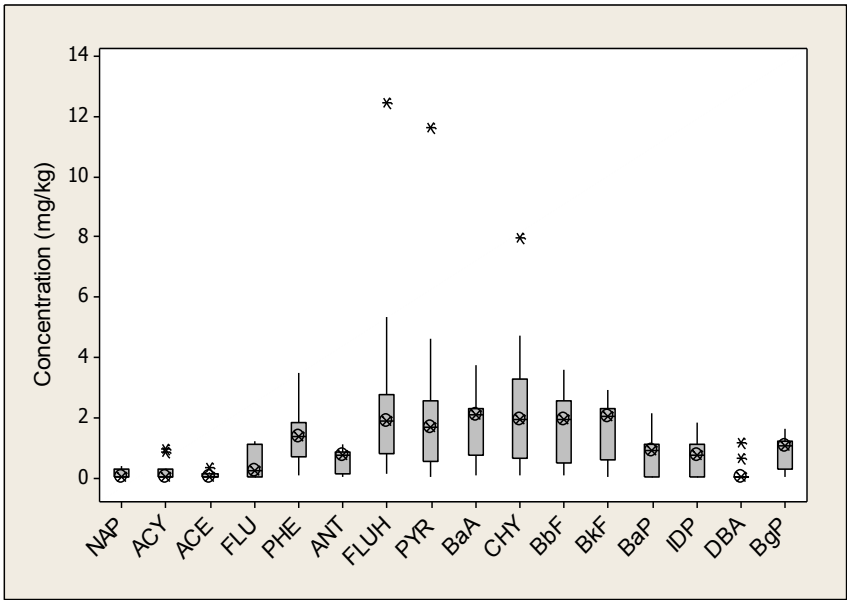


Figure 7.4: Box plot of individual PAH content of (all) dust samples (particle size > 250um) with interquartile range box, outlier symbols (*), median (cross) and whiskers indicated.

Identification of the source of PAHs has previously been investigated by the use of specific individual ratios to identify the proportion of pyrogenic and petrogenic PAHs in environmental matrices (Yunker *et al.*, 2002). The ratios used are anthracene / (anthracene + phenanthrene); fluoranthene / (fluoranthene + pyrene); benzo(a)anthracene / (benzo(a)anthracene + chrysene); and, indeno(1,2,3-cd)pyrene / (indeno(1,2,3-cd)pyrene + benzo(g,h,i)perylene) to determine the petrogenic or pyrogenic source of PAHs (Yunker *et al.*, 2002). The values of the ratios to estimate the proportion of each source are summarized in Table 7.5. By comparing these ratios with those calculated on our Newcastle dust samples (Figure 7.5), it appears that pyrogenic sources are predominant in our road dust samples, characteristic of vehicle exhaust emission.

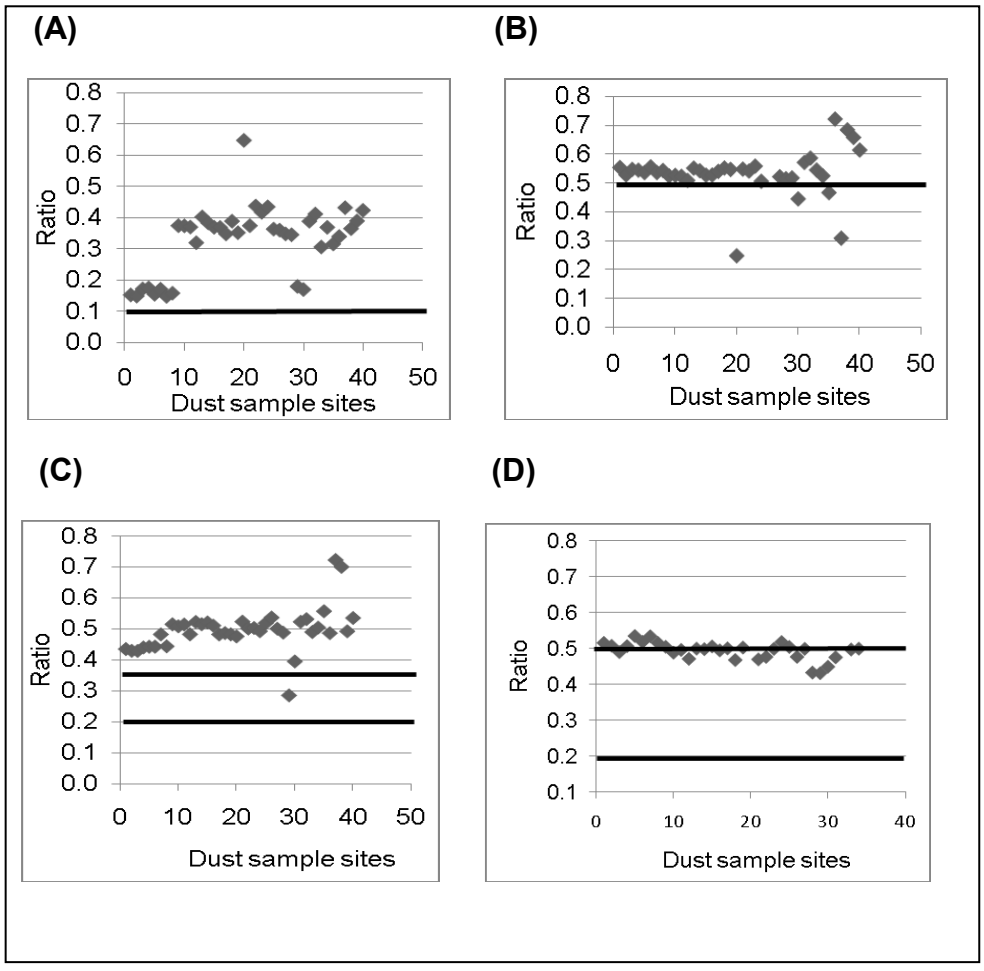


Figure 7.5: Source (petrogenic or pyrogenic) of PAHs in dust samples irrespective of particle size: (A) ANT / (ANT + PHE); (B) FLUH / (FLUH + PYR); (C) BaA / (BaA + CHY); and, (D) IDP / (IDP + BgP). The solid line represents the indicative discriminating ratios as noted in Table 7.5.

Table 7.5: Indicative ratios to distinguish petrogenic and/or pyrogenic sources of PAHs in roadside dust (Yunker *et al.*, 2002)

Ratio	Petrogenic source	Petroleum or combustion source	Pyrogenic source
ANT /(ANT+PHE)*	< 0.1		> 0.1
FLUH /(FLUH+PYR)*	< 0.5		> 0.5
BaA /(BaA + CHY)*	< 0.2	0.2-0.35	> 0.35
IDP /(IDP+ BgP)*	<0.2	0.2-0.5 (liquid fossil fuel combustion)	> 0.5

*cf. Table 7.2 for PAHs abbreviations

7.3.3 Organic matter influence

In order to explain variations in PAHs distribution between sampling sites, the organic matter content can be evaluated. The dust organic matter has a different composition than the soil organic matter, so caution needs to be taken when interpreting the results (Wang *et al.*, 2009). However, as demonstrated before in the chapter 5 for soils, the variations in dust organic matter do not show any correlation with the total PAH content (Table 7.6).

Table 7.6: Comparison of loss of ignition (%) with total PAH content for the 12 road dust sample sites at a particle size > 250 µm

Dust sample site (> 250 µm)	LOI %	Total PAH content
1	6.33	46.04
2	14.29	18.14
3	23.12	22.34
4	21.88	17.77
5	7.88	15.57
6	8.22	22.45
7	11.14	0.59
8	10.86	2.3
9	7.83	1.32
10	6.65	36.13
11	28.58	18.01
12	14.38	19.55

The same observations are made when comparing the variations in organic matter for three dust sampling sites, with a large range of particle sizes. The observations are

even contradictory with the common correlations usually observed in solid environmental matrices (Gron *et al.*, 2003; Mannino *et al.*, 2008), generally low organic matter content resulting in high PAH content. Indeed, with sample 10, the total PAH content is increasing as organic matter is increasing (Table 7.7). Therefore, in this site, the distribution of PAHs is not linked to the organic matter content. Furthermore, variations in total PAHs content in various particle sizes is possibly due to other parameters such as the surface area or the type of dust particles.

Table 7.7: Comparison of loss of ignition and total PAH content for three dust sample (10,11 and 12) sites with various particles sizes (0-63, 63-125, 125-250, 250-500,500-1000 and 1000-2000 µm)

	LOI %			**Total PAH content (mg/kg)**		
Road dust particle size	**10**	**11**	**12**	**10**	**11**	**12**
0-63 µm	18.02	NA	25.46	95.03	NA*	27
63-125 µm	9.45	24.94	21.26	51.35	28.71	24.89
125-250 µm	7.78	22.39	17.04	36.26	20.83	23.85
250-500 µm	6.65	28.58	14.38	36.13	18.01	19.55
500-1000 µm	8.40	32.23	19.63	40.12	22.1	19.35
1000-2000 µm	8.64	34.05	20.24	69.14	20.99	20.49

***Non available**

7.3.4 PAH distribution with respect to particle size

The total PAH concentration for three selected dust sampling sites (sites 10 – 12; i.e. samples with a large sample mass) in Newcastle-upon-Tyne city centre were investigated with respect to six particle size fractions (< 63 µm, 63-125 µm, 125-250 µm, 250 -500 µm, 500-1000 µm and 1000-2000 µm). For samples from sites 11 and 12, the distribution of total PAHs is independent of particle size investigated (Figure 7.6 (A)) but at sampling site 10 elevated concentrations are noted in two particle size fractions (i.e. < 63 µm and 1000-2000 µm). Moreover, when observing the variation of concentrations with two individuals PAHs (Figure 7.6 (B) and (C); phenanthrene and anthracene both showed elevated concentration in sample 10 for the largest particle size fraction (1000-2000 µm).

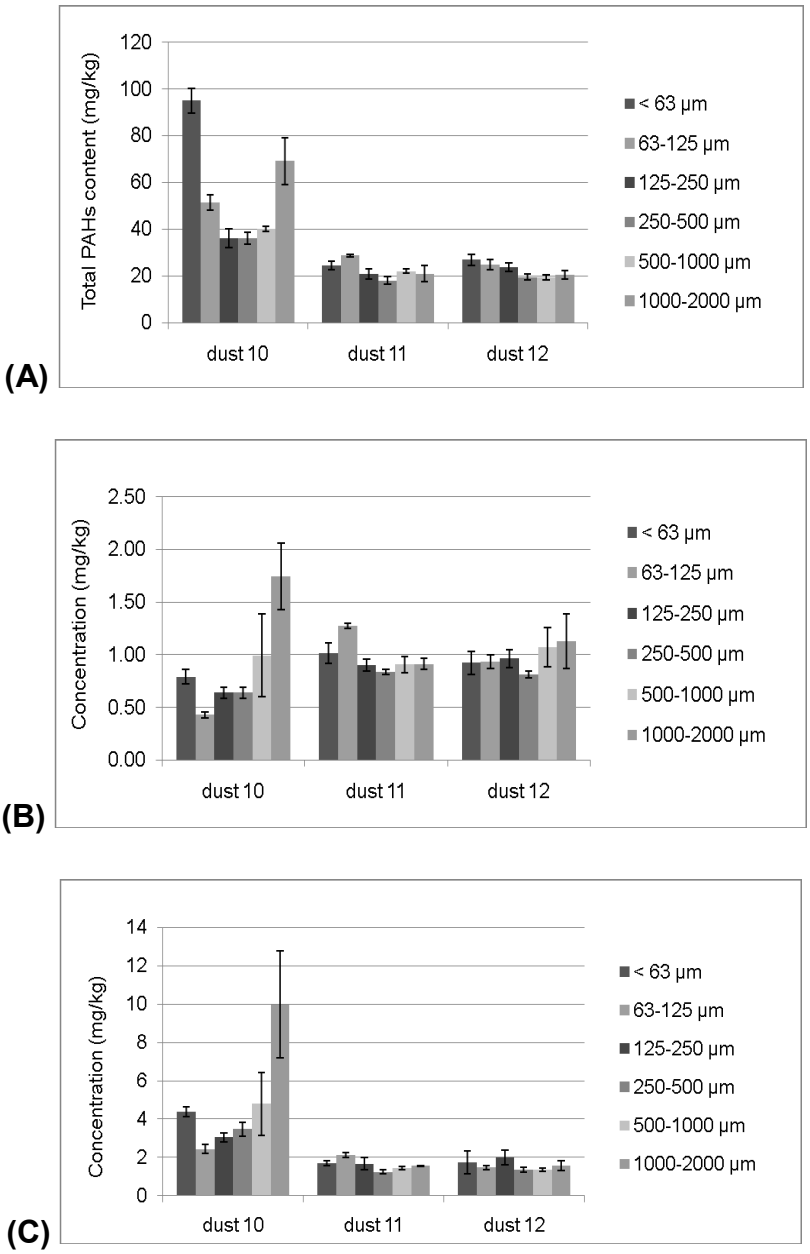


Figure 7.6. Investigation of PAH content (mg/kg) in three dust samples with respect to particle size (n = 3). (A) Total PAH; (B) Anthracene; and (C) Phenanthrene.

Further work is required on particle size variation of road dust samples, for the analysis of PAHs, in order to investigate the potential risk to human health from inhalation/ingestion. Moreover, the variation in concentrations between particle sizes can give further clues on the potential sources of PAHs in road dust. By observing the structure and colour of road dust particles, according to their particle size some conclusions can be drawn about variations in PAHs concentrations (Figure 7.6).

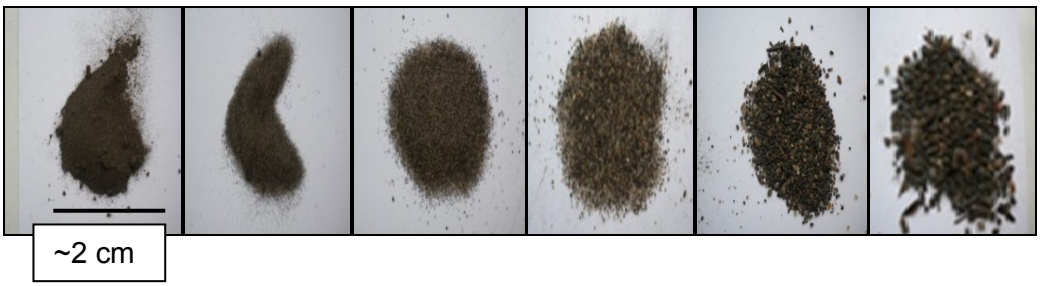


Figure 7.7: Pictures of a small amount of road dust for particle size 0-63, 63-125, 125-250, 250-500, 500-1000, 1000-2000 µm, sample 10 (from left to right).

Indeed, it appears that as particle size decreases, PAHs concentrations are getting higher for sample 10, and in the same way on the other extreme, where PAHs concentration are getting higher for bigger particle size of road dust . Sample 10 was chosen to realize this pictural comparison, as it contained the highest PAHs concentrations, so more confidence and more clarity was obtained with PAHs distributions (Figure 7.6 and 7.7). Several hypothesis could explain these variations.

Firstly the surface area of particles, which is getting higher at lower particle size can increase mobilization and attraction of PAHs into the particles of road dust. Secondly, the color could explain those differences, as a particle with a dark colour could prevent PAHs being degraded (Behymer *et al.*, 1988; Dong *et al.*, 2009), and darker colour is observed for both highest and lowest particle size. A final hypothesis would be the type of particles, which can change as particle size of road dust increases or decreases. Indeed, when observing Figure 7.7, the type and structure of particles is really different for the dust particles below 250 µm, which has the appearance of a powder, whereas for particle size above 500 µm particles have the shape of minuscule rocks, which could come from particles of pavement or tire debris, two known sources of PAH (Rogge *et al.*, 1993; Dong *et al.*, 2009; Wang *et al.*, 2009).

These differents ways of explaining those variations were explored in the literature. The high surface area of the finer grain size was increasing the adsorption of PAHs against the particles of dust, therefore increasing the concentration (Yang *et al.*, 1997; Fang *et al.*, 2004; Dong *et al.*, 2007; Dong *et al.*, 2009; Zhao *et al.*, 2009). This fact

could well illustrate the first trend where smaller particle size gives higher PAHs concentrations. A second theory to explain the high concentration of higher particle size is the effect of colour. The colour of a soil or dust particle is generally influenced by his content in organic matter. However, the organic matter seems to have no influence in the total PAH content of those dust samples, as well as for soils, demonstrated in this study. Therefore the colour and organic matter may not be the parameters leading to these variations.

Concerning the influence of the type of particles, one recent study has demonstrated, in the same way, that particles of asphalt were present at higher particle sizes from 850 to 2000 µm, thus increasing PAHs concentrations (Dong *et al.*, 2007; Dong *et al.*, 2009). Typical PAHs distributions in asphalt were also showing a higher concentration for phenanthrene and anthracene in the coarse grain size fraction (Takada *et al.*, 1991; Mostafa *et al.*, 2009), as observed in the present study, demonstrating that the type of dust particles can significantly influences PAHs distribution in various particle sizes of road dust. Therefore two main parameters could participate in the variation of PAHs distribution in road dust, within a range of particle sizes: the surface area and the type (or source) of dust particles.

7.3.5 Mean daily intake estimate of PAHs from urban dust and associated human health risk

The potential health risk from urban dust can be assessed by calculating the mass of dust that a child would be required to ingest to reach the estimated mean daily intake (MDI) for each individual PAH. The values of MDI are shown in Table 7.8 (Nathanial *et al.*, 2009).

Table 7.8: Oral PAH daily intake (µg) considering the involuntary ingestion of 100 mg/day* of dust

Compound	Intake of PAHs, based on maximum content from dust samples (n = 3), across 3 sites (site 10,11 and 12), through involuntary ingestion of 100 mg / day* of dust for a range of soil particle sizes (µg)						
	Soil particle size (µm)						
	0-63	63-125	125-250	250-500	500-1000	1000-2000	Oral MDI (µg/day) food+
	maximum (n = 3)	maximum (n = 3)	maximum (n = 3)	maximum (n = 3)	maximum (n = 3)	maximum (n = 3)	
Naphthalene	0.06	0.06	0.03	0.04	NA	0.05	7
Acenaphthylene	0.09	0.10	0.09	0.10	0.03	0.05	0.14
Acenaphthene	0.05	0.04	0.03	0.03	0.04	0.04	0.98
Fluorene	0.12	0.13	0.12	0.12	0.08	0.05	0.59
Phenanthrene	0.44	0.24	0.30	0.35	0.48	0.83	1.54
Anthracene	**0.10**	**0.10**	**0.10**	**0.08**	**0.11**	**0.14**	0.08
Fluoranthene	**1.35**	**0.67**	**0.55**	**0.53**	**0.62**	**1.00**	0.35
Pyrene	**1.09**	**0.60**	**0.42**	**0.46**	**0.49**	**0.90**	0.35
Benzo(a)anthracene	**0.82**	**0.50**	**0.37**	**0.37**	**0.39**	**0.54**	0.06
Chrysene	**1.06**	**0.66**	**0.49**	**0.47**	**0.49**	**0.60**	0.11
Benzo(b)fluoranthene	**1.28**	**0.65**	**0.34**	**0.33**	**0.36**	**0.43**	0.11
Benzo(k)fluoranthene	**0.61**	**0.38**	**0.29**	**0.29**	**0.31**	**0.31**	0.09
Benzo(a)pyrene	**0.91**	**0.45**	**0.24**	**0.21**	**0.23**	**0.32**	0.11
Indeno(1,2,3-cd)pyrene	**0.74**	**0.36**	**0.17**	**0.18**	**0.19**	**0.24**	0.10
Dibenzo(a,h)anthracene	**0.25**	**0.13**	**0.07**	**0.06**	**0.07**	**0.08**	0.04
Benzo(g,h,i)perylene	**0.70**	**0.35**	**0.19**	**0.16**	**0.20**	**0.21**	0.06

NA: not available

* Values based on the Child-Specific Exposure Factors Handbook, USEPA, September 2008;

+ Mean daily intake threshold for PAHs in food (Nathanial *et al.*, 2009); Figures in bold represent maximum individual PAH levels that exceed the stated oral MDI.

The US EPA (U.S Environmental Protection Agency, 2008) summarized studies of soil ingestion by children by several authors and has set guideline values for estimated soil ingestion rates at a mean value of 100 mg/day for children between the age 1 and 6 years. On that basis, the amount of individual PAH ingested from street dust has been calculated based on 100 mg per day. The values obtained were compared with the MDI values to assess the risk of each individual PAH to the child. From the results it is evident that all 4-6 membered ring PAHs i.e. fluoranthene to benzo(g,h,i)perylene, irrespective of particle size, exceed the MDI for a child based on involuntary ingestion. Though this calculation can help to estimate the potential risk from urban dust samples and highlights the important role of regular street sweeping activities, it is perhaps somewhat unrealistic because it only assumes ingestion (oral) as the means of exposure, and perhaps more importantly is based on a 100 mg daily intake of urban dust. However, some risk is noted that warrants further investigation, especially as the smaller particle size fractions i.e. <125 μm, can readily stick to hands and be unintentionally consumed by a child through hand-to-mouth contact.

7.4 Conclusion

The distribution of PAHs in samples of urban dust from Newcastle-upon-Tyne city centre indicates a potential risk to human health if quantities in excess of 100 mg/day are ingested, of any of the size fractions investigated across the 4–6 membered ring PAHs, either intentionally or unintentionally by hand-to-mouth contact based on published tolerable mean (oral) daily intakes. Our data also indicate that the maximum PAH concentrations are not consistently observed in the finer size fractions (i.e. <250 size fraction), and to what extent this is PAH (and source) specific is currently the focus of further investigation. In addition, it is reported that the dominant individual PAH in these urban dust are derived from pyrogenic sources with vehicle exhausts likely to be the main and dominant source. Indeed, the vehicle exhausts can be a source of PAHs in road

dust, depending on the vehicle speed. But the structure of the pavement and the tire debris are also a potential source of PAHs as described in the literature (Rogge *et al.*, 1993; Dong *et al.*, 2009; Wang *et al.*, 2009) and in this study. The pyrogenic sources seem to be predominant in either soil or dust from urban and industrial areas, as described in the present study and in the literature. Furthermore, as described for soil in a precedent chapter, the particle size is influencing the PAHs distribution inside the matrix, probably due to the surface area, and is of concern because of the potential ingestion of smaller particles via hand-to-mouth behaviour. In this particular chapter, the particles of dust were prepared at lower sizes than for soils, until 0-63 µm, meaning that further studies need to be realized with a larger range of particle sizes to consider the evolution of PAHs concentrations. Reaching very fine particle sizes (less than 10 µm) would involve the inhalation of those particles, which is another pathway of exposure, not explored in this study, but which implies also a risk for human health. Utilization of the value of 100 mg/day for the calculation of the daily intake of PAHs via involuntary ingestion is another way to consider the risk compared with the estimation of bioaccessibilities, and the direct comparison of total content with soil guideline values.

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Chapter 8: Conclusion and future work

8.1 Conclusion

This thesis has permitted to develop an efficient, robust, precise and accurate analytical procedure in order to analyse the 16 priority PAHs pollutants in solid environmental matrices. This method consists of using 2 g of alumina sorbent inside a cell integrated in a Pressurized Fluid Extraction system, called *in-situ* clean-up, and is followed by instrument analysis using a GC-MS.

This analytical procedure has therefore been applied to real samples. Firstly, this method has been used with soil samples from a former industrially contaminated site. The concentration of the PAHs in the soils demonstrated high concentrations, largely above currently available soil guidelines values for PAHs and values reported around the world. This site needs to be considered for remediation or any technique that would degrade the PAHs on site, to make the site clean and safe for the public, as it is situated close to people activities, near the Tyne River. Smaller particle sizes of those soils samples (<250 µm), which are more easily adhered on skin and ingested, have shown higher concentration of PAHs than with coarser grain sizes, which means that a higher risk will exist considering the ingestion exposure pathway.

As part of the study of the potential ingestion of solid environmental matrices, implementations of the Unified BARGE Method and the FORES(h)t in the present laboratory were realized and demonstrated good performance in terms of recoveries, precision and accuracy, using spiking procedures. The FORES(h)t did show satisfactory reproducibility as part of an interlaboratory study, demonstrating that this method is becoming robust as laboratories are implementing it in their laboratories. This fed state seems to be more appropriate and realistic when evaluating the risk from the ingestion of solid environmental matrices containing PAHs. Evaluation of PAHs bioaccessibilities

showed that the fed state was mobilizing significantly more PAHs than the fasted state, possibly due to changes in the chemical composition of the gastrointestinal fluids, notably the addition of food. The mechanisms of adsorption, absorption and mobilization inside the gastrointestinal tract are complicated but involve the formation of bile salts micelles which can attract hydrophobic compounds on their core, the similar attraction appearing with fat, both constituents influencing the adsorption of the hydrophobic constituents onto the cell walls of the intestine, therefore potentially entering systemic circulation and causing harm to human health. Other parameters can be influent in the mobilization of PAHs in the digestive tract such as the ring number, the molecular weight, the solubility and the partition coefficient of PAHs, but further studies are required as the mechanisms involved are complex and parameters could be in competition. It was noted that high total content can lead to low bioaccessibility, which is important when considering risk assessment. Based on the 100 mg/day ingestion rate and bioaccessible concentration, the risk was present the various soil samples, but not in the same proportion. For the Tar Works soils a third alternate way to estimate the risk was based on comparing total PAH content with soil guidelines values, which was also demonstrating another degree of risk for humans.

This analytical procedure developed, was used for the identification of PAHs in road dust samples from the city centre of Newcastle-upon-Tyne, as part of the examination of the risk involved via the ingestion exposure pathway, without the use of physiologically-based extraction tests. Concentrations were not as high as with the soil from the former industrial site, and they were in the same range of concentrations found around the world. Evaluation of PAHs content in a large range of particle sizes demonstrated possible variations in distributions due to the surface area of the particles and the sources of PAHs in an urban site, such as pavement and tire debris. Indeed, in the entire study, the organic matter did not show any correlations with the mobilization of PAHs in the solid

environmental matrices, therefore excluding this parameter, contrary to observations made in the literature. In some cases, PAHs concentrations were higher for finer particle sizes, which is again important considering the ingestion exposure pathway. The involuntary ingestion of 100 mg/day of soil per day for children, compared with the mean daily intake of PAHs in food, showed presence of risk with pyrogenic PAHs, in any of the particle size considered.

This study has shown that there are multiple ways to define the risk on a contaminated site. Bioaccessible fraction resulting from the FORES(h)t method test seems to be a realistic way to estimate and refine the risk *via* the ingestion exposure pathway, considering the analysis of PAHs in environmental matrices. The comparison of bioaccessible concentration or total PAHs content with the Mean Daily Intake is essential as it gives an information on the intake involved with the ingestion exposure pathway. However, variations in evaluations of the risks highlight that a consensus should be made on how to estimate the risk, and more particularly with the use of bioaccessibity.

8.2 Future prospects

The FORES(h)t method needs also further interlaboratory studies to finally enable it to be used in commercial laboratories. Development of certified reference materials for PAHs in bioaccessibility studies would also be essential, or any method that would control the quality of experiments made in various laboratories. Furthermore, ongoing production of new soil guideline values for PAHs and tools to evaluate the risk in UK by the Environmental Agency would help risk assessors and environmental scientists, to evaluate uniformly the risk throughout the country.

More studies should be done on the parameters that influence PAHs mobilization in soils, dust, and in the digestive tract, such as the surface area, the sources of PAHs, the molecular weight, the ring number, the solubility in water, the food and the soil-to-solution

ratio as mechanisms of attraction are complex. However, an understanding of the parameters that govern mobilization of PAHs will provide meaningful informations on the probability that has each individual PAHs (or group of PAHs) to be in contact with human and the environment, and therefore representing a risk.

The particle size parameter would need to be considered again and with a larger range of grain size to have a more accurate view on how the PAHs distributions can vary in a solid environmental matrix. This is particularly important as particle size is involved in the three different exposure pathways. More particularly, at very fine particle size, the inhalation pathway will be involved as particles of soils or road dust can become airborne. Further work considering this exposure pathway seems to be the way forward, as inhalation of pollutants in the environment can occur rapidly through human activities in urban areas. This pathway would however require a model simulating the respiratory tract.

GLOSSARY

AAS	Atomic Absorption Spectroscopy
BARGE	BioAccessibility Research Group of Europe
BGS	British Geological Survey
CLEA	Contaminated Land Exposure Assessment
CLR	Contaminated Land Report
CRM	Certified Reference Material
DCM	Dichloromethane
ED-XRF	Energy Dispersive X-ray Fluorescence
EI	Electron Impact
FL	Fluorescence
FID	Flame Ionization Detector
FORES(h)t	Fed Organic Estimation Human Simulation Test
GACs	Generic Assessment Criteria
GC-MS	Gas Chromatography-Mass Spectrometry
HCV	Health Criteria Values
HOC	Hydrophobic Organic Contaminants
HPLC	High Pressure Liquid Chromatography
IARC	International Agency for Research on Cancer
ICP	Inductively Coupled Plasma
ID	Index Dose
LC	Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification

MEPS	Micro Extraction by Packed Sorbent
MDI	Mean Daily Intake
PAHs	Polycyclic Aromatic Hydrocarbons
PBET	Physiologically Based Extraction Test
PCA	Principal Component Analysis
PCBs	Polychlorinated biphenyls
PDMS	PolyDimethylSiloxane
PFE	Pressurized Fluid Extraction
PLE	Pressurized Liquid Extraction
POPs	Persistent organic pollutants
PTV	Programme Temperature Vaporizer
RSD	Relative standard deviation
RPM	Revolution per minute
SBSE	Stir Bar Sorptive Extraction
SD	Standard Deviation
SFE	Supercritical Fluid Extraction
SGV	Soil Guideline Value
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
SSL	Split Splitless injector
TIC	Total Ion Current
TOF-MS	Time of Flight-Mass Spectrometry
TDI	Tolerable daily Intake
UBM	Unified BARGE Method
USEPA	United States Environmental Protection Agency